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Influence of Seasonal N Fertilization, Plant Growth Regulators, and Fungicide Application Timing on Anthracnose Severity of Annual Bluegrass Putting Greens

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B.S., Michigan State University, 2011

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Master of Science Thesis

Influence of Seasonal N Fertilization, Plant Growth Regulators, and Fungicide Application Timing on Anthracnose Severity of Annual Bluegrass Putting Greens

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ANTHRACNOSE

Anthracnose (caused by *Colletotrichum cereale* sensu lato Crouch, Clarke, and Hillman; Crouch et al., 2006) is a detrimental disease of cool-season (e.g., *Poa annua* L., *Agrostis* spp.) putting green turf in North America, the United Kingdom and Europe (Smiley et al., 2005). The occurrence and severity of anthracnose epiphytotics on annual bluegrass putting green turf significantly increased during the late 1990's and 2000's (Landschoot and Hoyland, 1995; Mann and Newell, 2005). Key factors purported to enhance anthracnose incidence included intensive cultural practices and environmental stress that weaken annual bluegrass (ABG) predisposing it to the pathogen (Vargas, 2005).

Symptoms and Signs

Symptoms of anthracnose appear as small bronze-yellow spots, whose size is approximately 1 cm (Vargas, 2005). As disease progresses, infected spots coalesce together and form large irregularly shaped areas of blighted turf (Smiley et al., 2005). On individual tillers, crown, stem and leaves are necrotic and chlorotic, with black, water-soaked basal rot. Under a microscope, saucer- or cushion-shaped fruiting bodies, called acervuli (Agrios, 2005) are found on infected plant tissue. Melanized, hair-like setae are unique structures used to identify the pathogen, produced within acervuli. Conidia of anthracnose produced within acervuli are hyaline, crescent-shaped single cell spores.

Epidemiology

Several *Colletotrichum* species are able to cause anthracnose diseases in cereals and grasses (Crouch et al., 2006). *Colletotrichum cereale* is the species which infects the Pooid grasses especially, creeping bentgrass (CBG; *Agrostis stolonifera* L.) and ABG (Crouch et al.,

2006). It inoculates hosts by spores, which produce appressoria to penetrate host cells. The rate of appressorial formation is strongly dependent on temperature (Wang and Kerns, 2015). On detached CBG and ABG leaves, when temperatures were 12°C, approximately 10% of conidia produced appressoria within 12 hours post inoculation (PI) and 90% within 48 hours in a controlled environment study. At 18 to 28°C, 80 to 100% of conidia formed appressoria within 24 hours PI. Appressorial formation was suppressed at 30 to 34°C with less than 40% of conidia producing appressoria within 96 hours PI.

Colletotrichum cereale has been speculated to exhibit a hemibiotrophic lifestyle throughout its interaction with turfgrass hosts (Crouch and Beirn, 2009). The hemibiotrophic pathogens in *Colletotrichum* species initially penetrate and colonize living host cells as a biotroph (Münch et al., 2008). During this asymptomatic phase, they rely on the nutrients transferred from living host cells (Mendgen and Hahn, 2002). During the necrotrophic phase, they grow secondary hyphae to penetrate adjacent host cells (Münch et al., 2008), and secrete toxins that destroy host tissues (Thines et al., 2006). The duration of the biotrophic phase of the *C. cereale*-host interaction, and the stimulus which induces the necrotrophic phase of the interaction are unknown.

Anthracnose can be detected on ABG at almost any time of year (Smiley et al., 2005). However, enhanced incidence and severity of anthracnose have been reported, when turfgrasses are under stressful condition. Heat stress, drought stress, poor drainage, soil compaction and traffic are purported to increase disease severity (Danneberger et al., 1995; Sprague and Evaul, 1930; Vargas, 2005).

ANNUAL BLUEGRASS

Turfgrasses that belong to the Pooideae are able to be infected by *C. cereale*, however, ABG is the most common host (Crouch et al., 2006). Annual bluegrass is a very diverse species throughout the world, often considered as a weed in golf course turfgrass. Annual bluegrass can invade CBG or other turfgrasses maintained as putting greens, and establish itself as a primary component of putting turf communities (Vargas and Turgeon, 2004). It is hard to eliminate because it has large a number of biotypes to survive under the selection pressure (Vargas and Turgeon, 2004).

Annual bluegrass is an allotetraploid species with 28 chromosomes (2n = 4x =28) and likely evolved as a result of a natural cross between two diploid species, *Poa infirma* H.B.K. and *Poa supina* Schrad. (Nannfeldt, 1937). Annual bluegrass is able to flower through the growing season; however, the main flowering period is in late spring and in summer. If a vegetative shoot is converted to a flowering shoot, it is impossible to produce any more leaf primordia (Vargas and Turgeon, 2004). New shoots arising from axillary buds or germinating seeds will replace the dead shoots. In general, producing seedheads utilizes carbohydrates or nutrients to support flowering culm and inflorescence development. Furthermore, root growth is suppressed due to the declining vegetative shoot and nutrient utilization of seedheads and new shoot growth (Kosky, 1983; Vargas and Turgeon, 2004). However, Ong and Marshall (1975) observed that removing seedheads could result in carbohydrates going downward to root system and to tillers. Annual bluegrass is able to tolerate a mowing height at 3.2 mm, to provide desirable putting green playability, and maintain acceptable quality. The ideal annual nitrogen (N) input on ABG green is between 195.2 and 292.8 kg N ha^{-1} to sustain plant health with a high ball roll distance (Vargas and Turgeon, 2004). Irrigation is essential to protect ABG from heat and drought stress.

Additionally, the loss of ABG in warm weather suggested ABG had poor heat and drought tolerance (Vargas and Turgeon, 2004). The new leaves grow and replace the dead inflorescences, resulting that the root growth of ABG is suppressed (Kosky, 1983; Vargas and Turgeon, 2004). Thus, ABG is unable to tolerate heat and drought stress due to the poor root growth in spring and summer.

CHEMICAL CONTROL OF ANTHRACNOSE

Fungicides are routinely applied to ABG putting green turf to prevent losses due to anthracnose. Applications are typically applied every 7 to 14 d from May to August. The total number of application per year may be anywhere from 8 to 16 depending on application interval and additional management practices. However, due to a lack of understanding of when primary infection by *C. cereale* occurs, an optimal timing for initiating fungicide applications is unknown (Tredway and Wong, 2012).

In a survey of 365 golf course superintendents from the United States, Canada and New Zealand during 2005 to 2007, the majority of respondents reported spending $10,000 to 40,000 year^{-1} on fungicides to manage turfgrass diseases on putting greens (Inguagiato and Kaminski, 2007, unpublished data). Of those reported fungicides, 21 to 30% were applied to control anthracnose. Fungicides within the chemical classes of nitrile, phenylpyrrole and demethylation inhibitor have generally provided acceptable anthracnose control in ABG putting greens across the United States (Tredway and Wong, 2012). However, tank mixtures of multiple fungicides containing two or more active ingredients, typically provided better disease control generally compared to applications consisting of a single active ingredient (Clarke et al., 2006; Inguagiato et al., 2014).

CULTURAL MANAGEMENT OF ANTHRACNOSE

Nitrogen Fertilization

Nitrogen is the most frequently deficient nutrient in nonlegume crop system (Havlin et al., 1999). It is the critical element of amino acids which are the basic components of protein. Nitrogen deficiency can decrease photosynthetic rate (Zhao et al., 2004), inhibit plant growth (Huber and Thompson, 2007), and reduce the tolerance to stressful environment (Orcutt and Nilsen, 2000). In order to prevent N deficiency in turfgrass systems, N fertilizer is routinely applied to turfgrass putting greens. Appropriate amount of N can enhance turfgrass yield, color, and visual quality in turfgrass systems. However, excessive N may cause excess turf shoot vertical growth, lead to low shoot density. Excessive N also can restrict turf root system growth and decrease root density (Murphy et al., 2005). Commonly, ABG putting greens require 195.2 to 292.8 kg N ha^{-1} per year for health and playability (Vargas and Turgeon, 2004).

Nitrogen fertilization effects on turfgrass disease have been shown in different studies. Williams et al. (1996) reported 73.2 kg N ha^{-1} reduced dollar spot (caused by *Sclerotinia homoeocarpa* F.T.Bennett) 57% on CBG green and 44% on fairways compared to no N input grass. Golembiewski and Danneberger (1998) reported that dollar spot severity decreased with elevated N levels. Application of 48.8 kg N ha^{-1} per month decreased dollar spot severity 14 to 65% on CBG putting green from July to September, while 24.4 kg N ha^{-1} per month reduced the severity 0 to 51%. The severity of red thread (caused by *Laetisaria fuciformis*) was reduced by increased annual N inputs from 98 to 291 kg ha^{-1} in perennial ryegrass (PRG; *Lolium perenne* L.; Cahill et al., 1983). The influences of N on anthracnose were reported by Danneberger et al. (1983). Danneberger et al. (1983) noted that 146 kg N ha^{-1} input per year reduced disease severity compared to 292 kg ha^{-1}. However, Inguagiato et al. (2008) demonstrated annual

application of 176.9 to 178.3 kg N ha^{-1} reduced anthracnose severity 5 to 24% compared to 90.3 to 93.8 kg N ha^{-1} on ABG putting green. Effect of seasonal timing of N fertilization was also tested by Danneberger et al. (1983). The particular mechanism of anthracnose severity reduction with moderate N fertility is not fully understood, although it has been speculated that the improvement of plant vigor by N fertility (White et al., 1978; Huber et al., 1987; Krupinsky and Tanaka, 2001) has potential benefit on anthracnose resistance of ABG (Inguagiato et al., 2008).

Plant Growth Regulators

Plant growth regulators (PGR) are organic compounds utilized in small amounts to promote, inhibit, or otherwise modify any physiological process in plants, without directly supplying either energy or mineral elements to the plant (Basra, 2000). Different kinds of PGRs are used to inhibit seedheads and vegetative growth, and enhance turfgrass quality as well (Murphy et al., 2005). For instance, ethephon, flurprimidol, mefluidide, paclobutrazol and trinexapac-ethyl are the common PGR for highly maintained turfgrass (Inguagiato, 2009a). Ethephon (ET; [(2-Chloroethyl) phosphonic acid]) is a plant growth regulator that releases ethylene in plant tissue (Foster et al., 1992). Ethylene is a classic phytohormone which can stimulate or inhibit elongation of root or shoot, regulate floral transition, and enhance environmental stress tolerance depending on the species and plant growth conditions (Vandenbussche and Van Der Straeten, 2012). Ethephon is able to inhibit inflorescence formation of grass crops, such as barley (*Hordeum vulgare* L.) or corn (*Zea mays* L.; Dahnous et al., 1982; Foster et al., 1992). It also had shown great ability to inhibit ABG seedhead production (Eggens et al., 1989; Kane and Miller, 2003; Haguewood et al., 2013). Annual bluegrass absorbs ET through both roots and shoots evenly, but redistributes from flag leaf to inflorescence most rapidly (McCullough and Sidhu, 2014; Sidhu et al., 2014). Although ET had negative effects on

warm-season grass turf quality and root growth, injury to ABG caused by ET was very weak (McCullough et al., 2005; Sidhu et al., 2014). The effect of ET on turfgrass disease is limited. Inguagiato et al. (2010) reported that two applications of ET at 3.8 kg a.i. ha^{-1} in late March and early April occasionally reduced anthracnose severity 3 to 22 % compared to no ET, while consistently decreasing seedhead.

Several studies have attempted different growing degree day (GDD) models to predict the application timing of ethephon to inhibit ABG seedheads. Growing degree day is a method to estimate plant growth and development via air temperature accumulation (McMaster and Wilhelm, 1997; Ritchie and NeSmith, 1991). Danneberger and Vargas (1984) reported a GDD model to spray mefluidide at 50 accumulate days starting 1 January with 10°C as the base temperature. Calhoun and Hathaway (2005) suggested a method that ET is initially applied between GDD93 and 260 with base temperature 0°C. It also stated that earlier application within this range controlled seedhead more effectively. In addition, a second application 21-day after the initial provided better results than a single application.

Trinexapac-ethyl (TE; [4-(cyclopropyl-α-hydroxy-methylene)-3,5-dioxocyclohexanecarboxylic acid ethylester]) is a widely used plant growth regulator, which inhibits the formation of gibberellic acid. In grass tissue, TE is converted to trinexapac acid that inhibits hydroxylation of GA20 to form GA1 by blocking the active of regulatory enzyme 3β-hydroxylase (Adams et al. 1992; Hedden 1991; Rademacher, 2000). GA1 controls the cell expansion and growth rate of the plant (Reid and Ross, 1991). Thus, application of TE to turfgrass reduces the GA1 concentration within grasses, and shoot growth is reduced.
It has been demonstrated that application of TE on PRG can reduce leaf elongation rate and improve tiller density (Ervin and Koski, 1998). It has also been reported that TE can improve

turf quality on both cool- and warm-season grass (Inguagiato et al., 2008; Lickfeldt et al., 2001; McCullough et al., 2006a). In addition, clipping yields of bermduagrass (*Cynodon dactylon* × *C. transvaalensis* Burtt-Davy), CBG (McCullough et al. 2007) and Kentucky bluegrass (KBG; *Poa pratensis* L.)were reduced by TE consistently (Lickfeldt et al., 2001; McCullough et al. 2007). However, the effect of TE on turfgrass root systems was not shown consistently. Ervin and Koski (1998) reported that TE had no influence on KBG root growth. McCullough et al. (2005; 2006b) demonstrated that TE treated bermudagrass had greater root mass and increased root length compared to nontreated turf or that treated with ET only. The reduction in rooting caused by ET was also masked by TE application (McCullough et al., 2005).

Stier and Rogers (2001) showed TE increased tiller density and enhanced color and chlorophyll content of supine bluegrass (*Poa supin*a Schrad.) under low irradiance condition. They also stated that clipping yield of the turfgrass was reduced by TE application. Furthermore, turf quality, density, and chlorophyll levels were improved in TE treated CBG, KBG under 60% to 80% shade (Goss et al., 2002; Steinke and Stier, 2003). Richardson (2002) observed TE applied with late growing season N resulted in longer fall green color retention of bermudagrass and earlier spring green-up. Golembiewski and Danneberger (1998) reported TE reduced dollar spot severity in CBG fairway turf and occasionally override the effect of different levels of N. It has been speculated that suppression of turfgrass leaf growth could result in N reallocation in TE treated grass tissues. Fagerness et al. (2004) determined that TE decreased N allocation to clippings by approximately 25% and improved N moving to root system and rhizomes in bermudagrass turf. Further study demonstrated that cytokinin content in CBG, KBG and bermudagrass is increased by application of TE (Ervin and Zhang, 2007). They speculated that demand for shoot growth or leaf elongation was reduced by TE, and thus carbohydrates and

nutrients were transported to basal sink tissue (i.e., roots). Improved root metabolism may result in increased cytokinin biosynthesis, which could be transported back to upper tissues (Ervin et al., 2007). Greater cytokinin content of crown could regulate and induce more tiller formation. Additionally, in combined drought and heat stress studies, turf quality, shoot growth, canopy photosynthetic rate and foliar chlorophyll content of CBG were enhanced by TE application, compared to non TE treated turf (McCann and Huang, 2007).

The positive effects of TE might coordinate the maintenance of turfgrass. However, the activity of TE in plants is directly related to air temperature and the metabolism rate was doubled by doubled air temperature (Beasley and Branham, 2005). Two studies observed the reduction of growth suppression duration affected by temperature with TE(Beasley et al., 2007; Fagerness et al., 2002; Lickfeldt et al. 2001). Increased temperature accelerated the dissipation of TE, and caused less suppression duration. In contrast, TE catabolism decreased at lower temperature with greater growth suppression. To predict when TE needs to be applied, a GDD model was built by Kreuser and Soldat (2011). They found that reapplication of TE every 200 GDD with a base of 0°C is the optimum model to maintain growth suppression of CBG. There was no significant difference observed between 0.05 and 0.10 kg a.i. ha^{-1} throughout the duration of the study. Kreuser and Soldat (2012) also demonstrated that TE applied by 200 GDD at 0.10 kg a.i. ha^{-1} reduced clipping yield and N removal from CBG.

The effect of TE on anthracnose severity of ABG had been reported in recent years. Trinexapac-ethyl applied at 0.05 kg a.i. ha^{-1} every 2 week interacted with mefluidide applied at 0.106 kg a.i. ha^{-1} twice in April reduced anthracnose severity compared to turfgrass receiving only one of them (Inguagiato et al., 2008). However, the reduction of TE only was not consistent in previous studies, while the effects of greater TE rate (0.05 vs. 0.8 kg a.i. ha^{-1}) and shorter

application interval (7 vs. 14d) on decreasing anthracnose severity were occasional (Inguagiato et al., 2008; 2009a; 2010). A speculation was noted by Inguagiato et al. (2010) that TE had the greatest effect on anthracnose severity in the year with additional 31.7 kg spring N ha^{-1}. Trinexapac-ethyl might improve the utilization of additional spring N and reduced disease severity more effectively.

Prohexadione-Ca (calcium;1-(4-carboxy-2,6-dioxocyclohexylidene)propan-1-olate; PC) is another GA inhibitor newly registered for use in turfgrass under the trade name Anuew (Nufarm, Morrisville, NC). Prohexadione-Ca has a similar mechanism as TE, to inhibit GA1 synthesis in plant tissues (Rademacher, 2000). The biosynthesis pathway of GA1 was blocked by PC resulting in alleviated seedling elongation rate and reduced height of rice (*Oryza sativa* L; Na et al., 2011; Nakayama et al., 1990). Previous studies also showed that PC reduced vegetative growth of cherry (*Prunus avium* L.), peanut (*Arachis hypogaea* L.), apple (*Malus domestica* Borkh.) and turfgrasses, including KBG, PRG, bermudagrass and zoysiagrass (*Zoysia japonica* Steud. ;Beam, 2004; Elfving et al., 2003; Mitchem et al., 1996; Unrath, 1999). Obear and Kreuser (2014) demonstrated that PC suppressed CBG growth at a similar level compared to TE. However the duration of PC growth regulation on CBG putting green was approximately 50% longer than TE. hey suggested that the ideal interval of PC application would be 300 GDD with base temperature of 0°C. Since PC has a similar mechanism with TE to suppress turfgrass growth, PC may also impact anthracnose severity on ABG putting green.

Mowing height

Mowing is the most basic and important cultural practice on golf course putting green, fairways and tees to provide playability. Lower mowing height would result in excess shoot tissue removal and reduction of photosynthesis. Additionally, the lower mowing height could

cause reduction of root depth, and decrease the stress resistance of turfgrass (Williams and McCarty, 2005). Anthracnose severity has been demonstrated to increase as mowing height is reduced within heights commonly used for putting greens (i.e., 2.8, 3.2, or 4.1 mm; Inguagiato et al., 2009b). Even small changes in bench settings (0.4 mm) at common mowing heights was sufficient to result in significant change in disease severity (Inguagiato et al., 2009b).

Verticutting

Verticutting (VC) is a management practice used to remove excess thatch or non-uniform shoot density on sports fields and golf course fairways and putting greens (Barton et al., 2009; Stier and Hollman, 2003). It was reported that VC to a 5-mm depth increased anthracnose severity on a mixed ABG and CBG putting green compared to a 3-mm or no VC (Uddin and Soika, 2003); whereas, Inguagiato et al. (2008) observed little effect of a 3-mm VC on decreasing disease.

Lightweight Rolling

Lightweight rolling is a maintenance practice providing benefits on greens' playability by increasing uniformity and ball roll distance (Hartiwiger et al., 2001; Inguagiato et al., 2009b; Nikolai, 2005). It has also been observed that rolling decreased dollar spot incidence (Nikolai et al., 2001) and severity (Giordano et al., 2012) on CBG and ABG putting green. Inguagiato et al. (2009b) reported that rolling ABG putting green every other day with a triplex-attached vibratory roller slightly reduced anthracnose severity under moderate disease pressure, but had no effect under severe disease pressure.

Topdressing

Topdressing can help dilute thatch accumulation, smooth playing surfaces, encourage turf density and modify soil by adding a thin layer of soil to the turf surface (Murphy et al., 2005).

Inguagiato et al. (2012) demonstrated that 0.3 or 0.6 L sand m^{-2} applied every 7 or 14 d reduced anthracnose severity on annual bluegrass putting green, and relatively high sand rates (1.2 L m^{-2}) applied at longer intervals (21 to 42 d) also reduce disease severity but required longer time to produce the result. It has also been suggested that there was a critical cumulative amount of sand required (2.4 to 4.8 L m^{-2} in 2006) to reduce anthracnose severity compared to non-topdressed turf. Hempfling et al. (2015) noted that spring topdressing decreased the cost and need of summer topdressing for anthracnose control, although the effect of summer topdressing could not be ignored. It was speculated that the mat layer formed by topdressing sand protected turf from environmental stresses occurring at the surface and low mowing height (Hempfling et al., 2015; Inguagiato et al., 2012).

Irrigation

Irrigation is necessary to maintain high performance turfgrass, and also prevent potential drought stress. The reported effects of irrigation on anthracnose on ABG were various (Beard et al., 1978; Sprague and Evaul, 1930). Roberts et al. (2011) studied irrigation quantity based on reference evapotranspiration effects on anthracnose on ABG. It was noted that 80% reference evapotranspiration provided enough water to maintain satisfactory growth of ABG turf and limit anthracnose severity with avoiding dry soil or algae development.

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CHAPTER 1. Effect of Temperature Threshold Based Fungicide Application Timing on Anthracnose Severity of Annual Bluegrass Putting Green Turf

ABSTRACT

Anthracnose (caused by *Colletotrichum cereale* sensu lato Crouch, Clarke, and Hillman) is a detrimental disease of annual bluegrass (ABG; *Poa annua* L.) putting greens. Fungicides are generally used to control anthracnose on putting green turf. However, the estimation of fungicide initial application date is not accurate. The infection by *C. cereale* is facilitated by formation of an appressorium to penetrate a host cell. It has been demonstrated that the appressorial formation speed and rate is temperature dependent. A two-year field study was established in 2014 to evaluate the effect temperature threshold based fungicide application timing on anthracnose severity of ABG putting green turf. Temperature threshold of 10, 14, 18 and 22°C were selected to compare with conventional fungicide initial application timing. No difference between conventional application timing and temperature threshold of 10, 14, and 18°C was observed. Temperature threshold of 22 °C resulted in greater anthracnose severity during 2014. However, it reduced disease severity during late July and early August 2015.

INTRODUCTION

Anthracnose (caused by *Colletotrichum cereale* sensu lato Crouch, Clarke, and Hillman; Crouch et al., 2006) is a devastating disease of cool-season (e.g., *Poa annua* L. and *Agrostis* spp.) putting green turf. Symptoms initially appear as small, bronze-yellow spots, approximately 1 cm in diameter (Vargas, 2005). As disease progresses, infected spots coalesce and form large irregularly shaped areas of blighted turf on putting greens (Smiley et al., 2005). On individual plants, stem and leaf tissues are often chlorotic, turning necrotic and crowns may exhibit a dark, water-soaked rot. Signs of disease include saucer- or cushion-shaped fruiting bodies, called acervuli found on infected plant tissue (Agrios, 2005). Melanized, hair-like setae are unique

structures, produced within acervuli, used to identify the pathogen. *Colletotrichum cereale* produces single-celled, hyaline, crescent-shaped conidia.

Cultural practices are known to have a significant impact on anthracnose severity of annual bluegrass (ABG) turf. Nitrogen applied every 7 d at 4.9 kg ha^{-1} during the summer has been demonstrated to reduce anthracnose severity compared to a 28 d interval on ABG putting greens (Inguagiato et al. 2008). Inguagiato et al. (2009b) observed that increasing mowing height from 2.8 to 3.6 mm reduced anthracnose severity on ABG putting green turf. Plant growth regulators ethephon [(2-Chloroethyl) phosphonic acid], trinexapac-ethyl (TE; [4-(cyclopropyl-α-hydroxy-methylene)-3,5-dioxocyclohexanecarboxylic acid ethylester]) and mefluidide (N-[2,4-dimethyl-5-(trifluoromethylsulfonylamino)phenyl]acetamide) reduced disease severity occasionally in previous researches (Inguagiato et al., 2008; 2009a; 2010). Topdressing has been identified to reduce anthracnose severity depending on seasonal application (Inguagiato et al., 2012; Roberts and Murphy, 2014; Hempfling et al., 2015;).

Fungicides are widely used to control anthracnose on ABG putting green turf. Based on conversations with golf course superintendents, fungicides for anthracnose control are initially applied in May prior to the symptom showing up on ABG putting greens. Between May and August, fungicides may be applied at an interval of every 7 to 14 d. The total number of application per year may vary from 8 to 16 times. Because of a lack of understanding of the disease cycle of *C. cereale*, initial fungicide application timing is based on preceding the historical onset of symptoms for a given area. However, it is possible that infection by *C. cereale* may have already by this time since the time of primary infection is poorly understood (Tredway and Wong, 2012). In a survey of 365 golf course superintendents from the United States, Canada, and New Zealand during 2005 to 2007, the majority of respondents reported

spending $10,000 to 40,000 year^{-1} on fungicides to manage turfgrass diseases on putting greens (Inguagiato and Kaminski, 2007, unpublished data). Of those reported fungicides, 21 to 30% were applied to control anthracnose. Fungicides within the chemical classes of nitrile, phenylpyrrole and demethylation inhibitor have generally provided acceptable anthracnose control in ABG putting greens across the United States (Tredway and Wong, 2012). However, tank mixtures of multiple fungicides containing two or more active ingredients, generally provided better disease control compared to applications consisting of a single active ingredient (Clarke et al., 2006; Inguagiato et al., 2014).

Colletrotrichum cereale has been speculated to exhibit a hemibiotrophic lifestyle throughout its interaction with turfgrass hosts (Crouch and Beirn, 2009). The hemibiotrophic pathogens in the *Colletotrichum* genera initially penetrate and colonize living host cells as a biotroph (Münch et al., 2008). During this asymptomatic phase, they rely on the nutrients acquired from living host cells (Mendgen and Hahn, 2002). In the necrotrophic phase, they grow secondary hyphae to penetrate adjacent host cells (Münch et al., 2008), and secrete toxins that destroy host tissues (Thines et al., 2006). The duration of the biotrophic phase of the *C. cereale*-ABG interaction, and the stimulus which induces the necrotrophic phase of the interaction are unknown. However, it is possible that *C. cereale* infects turfgrasses days or weeks before symptoms develop. Thus, it may be possible that current recommendations for initial fungicide applications may not be applied at a time to prevent primary infection from occurring.

Primary and secondary infection by *C. cereale* is facilitated by formation of an appressorium to penetrate a host cell. Wang and Kerns (2015) demonstrated that appressorial formation in *C. cereale* is temperature dependent on detached creeping bentgrass and ABG leaves. When temperatures were 12°C, approximately 10% of conidia produced appressoria

within 12 hours post inoculation (PI) and 90% within 48 hours in a controlled environment incubator. At 18 to 28°C, 80 to 100% of conidia formed appressorial within 24 hours PI. Appressorial formation was suppressed at 30 to 34°C with and less than 40% of conidia producing appressoria within 96 hours PI. Wang and Kerns (2015) hypothesized that disease severity and fungicide use may be decreased, if an initial fungicide application was made prior to appressorial formation. In southern New England (Hartford, CT; Storrs, CT; Amherst, MA; Boston, MA; Providence, RI), monthly average temperatures are 8.9, 14.5, 19.6, 22.5 and 21.72°C in April, May, June, July and August, respectively (National Weather Service, 1981-2010). The timing of fungicide application would be critical and important to control anthracnose for improving effectiveness and efficiency of fungicide and reducing the cost. Therefore, the objective of this study was to evaluate the effect of temperature threshold based fungicide application timing on anthracnose severity of ABG putting green turf.

MATERIALS AND METHODS

Site Description

The two-year field study was initiated in 2014 on a 5-yr old ABG putting green turf grown on a Paxton fine sandy loam (coarse-loamy, mixed, active, mesic Oxyaquic Dystrudepts) with a pH of 6.2 at the University of Connecticut Plant Science Research and Education Facility in Storrs, CT. The field was established on a previously existing mixed creeping bentgrass and ABG putting green derived from ABG sods and soil cores from golf courses throughout CT. Prior to initiating the study, creeping bentgrass (*Agrostis stolonifera* L.) was eliminated with fluazifop-P-butyl {Butyl (R)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoateat} 0.05 kg a.i. ha^{-1} on 16 May 2009. The field was core cultivated and additional soil cores from

ABG putting greens obtained from Wethersfield Country Club in Wethersfield, CT were topdressed uniformly across the field. All cores were pulverized with a verticutter and re-distributed with a box-link drag. Creeping bentgrass was eliminated from the site with fluazifop-P-butyl (Butyl (R)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoateat) 0.05 kg a.i. ha^{-1} on 16 May 2009. A monostand of ABG maintained at 3.2 mm was established by June 2010. Anthracnose developed uniformly throughout the study area each year from a naturally occurring infestation of *C. cereale*.

The field was mowed 5 days wk^{-1} at a bench setting height of 3.2 mm with a walk behind mower (Model PGM 22, Jacobsen Textron, Charlotte, NC). Nitrogen (water soluble sources) was sprayed to the field uniformly to sustain turf vigor and encourage recovery from disease damage suffered by the previous year during April through October at 62.85 kg ha^{-1} in 2014 and 69.59 kg ha^{-1} in 2015. Spring N applications were 25.82 kg ha^{-1} or 24.7 kg ha^{-1} on April and May 2014 and 2015, respectively. Summer N was applied at 2.24 kg ha^{-1} biweekly or 2.24 weekly on June through August 2014 and 2015, respectively, when the total summer N were 14.59 and 28.1 kg ha^{-1}. During fall 2014 and 2015, N was applied at 22.45 and 16.8 kg ha^{-1}. Sand topdresssing was applied at approximately 0.2 L m^{-2} and incorporated with a coco-mat drag every 14-d from May to September each year. Irrigation was applied to the field as needed to avoid drought stress.

Dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett) was preventively controlled in the study area from May through August in 2014 and June through August in 2015 with vinclozolin [3-(3, 5-dichlorophenyl)-5-ethenyl-5-methyl-2, 4-oxazolidinedione] at 1.5 kg a.i. ha^{-1}, boscalid {3-pyridinecarboxamide, 2-chloro-N-[4'-chloro(1,1'-biphenyl)-2-yl]} at 0.4 kg a.i. ha^{-1} and May in 2014 and Fluazinam {[3-chloro-N-[3-chloro-2,6-dinitro-4-trifluoromethyl)phenyl]-5-trifluoromethyl-2-pyridinamine]} at 0.6 kg a.i. ha^{-1} every 14-d. Flutolanil {N-[3-(1-

methylethoxy) phenyl]-2-[trifluoromethyl] benzamide} at 3.2 to 6.4 kg a.i. ha^{-1} during June and July 2014 and Fluazinam {[3-chloro-N-[3-chloro-2,6-dinitro-4-trifluoromethyl)phenyl]-5-trifluoromethyl-2-pyridinamine]} at 0.6 kg a.i. ha^{-1} in July 2015 were applied to control brown patch (*Rhizoctonia solani* Kuhn). Algae was suppressed by zinc and manganese ethylenebisdithiocarbamate { [2-(dithiocarboxyamino)ethylamino]-sulfoniumylidenemethanethiolate; zinc; manganese}applied once $month^{-1}$ at 23.8 kg a.i. ha^{-1} from June to August 2014. Downy mildew (*Sclerophthora macrospora* (Sacc.) Thirum) and Pythium blight (*Pythium aphanidermatum* (Edson) Fitzp.) controlled with applications with mefenoxam {(R,S)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester}at 0.7 kg a.i. ha^{-1} on 29 Apr 2014 and 9 Jul 2015. The anthracnose epiphytotic was arrested between study years with applications of polyoxin D zinc salt { zinc 5-[[2-amino-5-O-(aminocarbonyl)-2-deoxy-Lxylonoyl]amino]-1-(5-carboxy-3,4-dihydro-2,4- dioxo-1(2H)-pyrimidinyl)-1,5-dideoxy-β-Dallofuranuronate} at 0.3 kg a.i. ha^{-1} and chlorothalonil (tetrachloroisophthalonitrile) at 8 kg a.i. ha^{-1} to promote turf recovery. Annual bluegrass weevil [*Listronotus maculicollis* (Kirby)] adults and larvae were eliminated respectively with lambda-cyhalothrin [1α(S), 3α(Z)]-(±)-cyano(3-phenoxybenzyl)methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2,-dimethylcyclopropanecarboxylate} at 0.07 kg a.i. ha^{-1}, Dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate at 9.1 kg a.i. ha^{-1} in May 2014 and Chlorantraniliprole {5-bromo-N-[4-chloro-2-methyl-6-(methylcarbamoyl)phenyl]-2-(3-chloropyridin-2-yl)pyrazole-3-carboxamide} at 0.2 kg a.i. ha^{-1} in June 2015.

Treatment and Experimental Design

The study was conducted as a randomized complete block design with four replications. Chlorothalonil (Daconil Ultrex 82.5% a.i., Syngenta Crop Protection, Greensboro, NC) was

initially applied at 8 kg a.i. ha^{-1} when average daily air temperatures reached 10, 14, 18 and 22 °C for 3 consecutive days on 1 May, 12 May, 12 May, and 2 Jul 2014; and 4 May, 5 May, 12 May, and 13 Jul 2015. Temperature data were collected from an onsite weather station. Chlorothalonil was applied at conventional application timing at the same rate on 2 Jun 2014 and 18 May 2015. Treatments of 10, 14, 18 °C and conventional method were applied every 7-d through 1 Jul 2014 and 26 May 2015. Last applications of 22°C treatment were on 11 Jul 2014 and 20 Jul 2015. Additionally, an untreated control was added into the experiment. All treatments were applied using a hand-held CO_2 powered sprayer with a single AI9508E flat fan nozzle (TeeJet Technologies; Glendale Heights, IL) calibrated to deliver 814 L ha^{-1} at 276 kPa.

Data Collection and Analysis

Anthracnose severity was assessed as the percent plot area blighted by *C. cereale* every 7-d from July through early August of 2014 and 2015 using a line intercept grid count method described by Inguagiato et al. (2008) during July and early August of 2014 and 2015. Area under the disease progress curve (AUDPC) values were calculated based on percent plot area blighted to assess treatment effects throughout the epiphytotic. All data were subjected to analysis of variance using the MIXED procedure in the Statistical Analysis System software v. 9.4 (SAS Institute Inc., Cary, NC). Treatment means were separated using Fisher's Protected Least Significant Difference test (p=0.05).

RESULTS

The average air temperature for April 2014 was similar to April 2015 (Fig.1). However, conditions required to meet the 10°C threshold (i.e., 3 consecutive days with average air temperature ≥ 10°C) were achieved by 14 Apr 2014, but not until 4 May 2015 (Table 1). Cool

temperatures during late-April and early-May 2014, prevented initiation of subsequent treatments until 12 May, 28 d after the initial 10°C applications were made. Once temperatures increased, thresholds for both 14 and 18°C treatments were reached on the same day (12 May). Conversely, higher temperatures persisted in early-May 2015. As a result, threshold for 14 and 18°C application timings were met within 8 days of the initial 10°C treatment in 2015. Average air temperature for late-June to mid-July were 22°C and 20°C for 2014 and 2015, respectively. Threshold treatments associated with the 22°C application timings were applied early- to mid-July during the two years.

Anthracnose developed from a natural infestation throughout the study area on 2 July and 7 July, in 2014 and 2015, respectively. During 2014, disease progressed rapidly in untreated turf during July, reaching the peak of the epidemic on 28 July, with 75% plot area blighted (Fig. 2). Thereafter disease decreased throughout August as overnight temperatures subsided. During 2015, anthracnose severity was considered moderate in untreated turf through mid-July (16 July; Fig. 3). However, in late-July and early-August, disease severity increased to a maximum of 49.6% plot area blighted.

Anthracnose Severity 2014

During, 2014, significant differences were detected among treatments on all evaluation dates (5 of 5 dates; Table 2). As anthracnose initially began to develop on 2 July, treated turf corresponding with 10°C, 14°C, 18°C threshold and conventional fungicide timings all reduced disease severity approximately 13% compared to untreated control and 22°C threshold timing plots (Fig. 2). No differences ($p > 0.05$) between the 10°C, 14°C, 18°C or conventional treatments were observed at this time; which was one day after each received a final fungicide application 1 July. No difference between 22°C threshold and untreated control were observed

on this date either, although the temperature threshold for this treatment had not been met by this time, therefore no fungicide had been applied to these plots prior to this date (2 July; Table 1). Anthracnose severity increased in all treatments throughout July, with untreated plots reaching 75% plot area blighted by the end of the month. However, 10°C, 14°C, 18°C and conventional treatment timings continued to reduce disease 42 to 64% compared to the untreated control during July. Anthracnose severity in these plots was 6 to 15% of the total plot area blighted throughout July; up to 27 days after the last chlorothalonil application. No differences between these initial fungicide timings (i.e., 10°C, 14°C, 18°C and conventional timings) were observed during this time. The temperature threshold for the 22°C timing was met on 2 July and an additional chlorothalonil application was made to these plots on 11 July (Table 1).

The initial application of chlorothalonil at the 22°C threshold timing suppressed diseased development within 7 days, reducing anthracnose severity 10% compared to the untreated control on 11 July. The two chlorothalonil applications associated with the 22°C threshold timing continued to suppress anthracnose severity 16 to 19% compared to the untreated control throughout July, up to 17 days after treatment. However, disease severity of 22°C threshold timing treatment remained 12 to 49% greater than 10°C, 14°C, 18°C, and conventional timings during July.

Anthracnose severity decreased in all treatments by the last observation date on 4 August. Significant treatment differences in AUDPC values were detected (Table 2). Area under the disease progress curve values for the 10°C, 14°C, 18°C, and conventional timing were reduced 83.0 to 86.4% compared to untreated control. No differences between these timings (i.e., 10°C, 14°C, 18°C, and conventional timings) were observed. The AUDPC for the 22°C threshold

timing was 24% less than the untreated control, however, it was 3.5 to 4.6 times greater than 10°C, 14°C, 18°C and conventional timings.

Anthracnose Severity 2015

All treatments contained 11 to 21% plot area blighted by anthracnose during the onset of symptoms on 7 July (Fig. 3). No differences were observed among treatments on this date, 43 days after the last chlorothalonil application for 10°C, 14°C, 18°C and conventional treatment timings, and 6 days before initiation of the 22°C threshold timing treatment (Table 2). Anthracnose severity increased slightly in all treatments by 16 July, and small differences between treatments had become evident (Fig. 3). The only treatments that reduced anthracnose severity compared to the untreated control at this time were plots initially treated at 10 and 14°C.

Initial application timing for the 10 and 14°C temperature thresholds were met within one day of each other (i.e., 4 and 5 May, respectively) during 2015 (Table 1). Thus, these two treatments contained similar amounts of disease at this time. The 10°C threshold timing had 4 to 10% less disease than 18°C, 22°C, conventional timings, and the untreated control. However, the 14°C threshold timing reduced anthracnose severity 5 to 7% compared only to the 22°C threshold timing and the untreated control, but was no different from 10°C, 18°C and conventional treatment timings. No significant differences between 18°C, conventional treatment timings and untreated control were detected on this date.

The temperature threshold for the 22°C treatment was met on 13 July (Table 1), although no difference in anthracnose severity was observed compared to untreated control on 16 July, 3 days after treatment. By 23 July, disease severity had increased 7.4 to 13.8 times in all treatments compared to plots treated at the 22°C threshold timing. The 22°C threshold treatments had

received 2 chlorothalonil applications by this date, with the last occurring 3 days earlier; whereas all other treatments had not received an application of chlorothalonil for 59 days.

No differences were observed between 10°C, 14°C, 18°C, conventional treatment timings or untreated control on 23 and 29 July. On the final observation date (5 August) the 22°C threshold treatments continued to have 7 to 26% less plot area blighted compared to all other treatments, 16 days after treatment. The 10 and 14°C threshold timings reduced disease 5 to 9% compared to the 18°C, conventional treatment timings and untreated control on 5 August, although turf treated at 18°C, conventional treatment timings and untreated control all had similar amounts of disease.

Significant differences of AUDPC values were found among treatments (Table 3). The 22°C threshold treatment had the AUDPC value during 2015, and was 20 to 31% less compared to 14°C, 18°C, conventional timings and untreated control (Fig. 3). Turf initially treated at the 10°C threshold did not differ from other treated turf, but was 18% less than the untreated control. No differences were observed among all other treatments.

DISCUSSION

Wang and Kerns (2015) speculated that fungicide application initiated before temperatures reach the optimal range for *C. cereale* appressorial formation could potentially inhibit infection and prevent anthracnose. Thus, temperature threshold for initial fungicide application selected in this study (i.e., 10°C, 14°C, 18°C and 22°C), were lower than the optimum temperature range of *C. cereale* appressorial formation (i.e., 22°C to 28°C), reported by Wang and Kerns (2015), and were intended to prevent primary infections. Results from this two-year study did not support the hypothesis that early fungicide application may prevent primary

infection by *C. cereale*, and reduce the need for fungicide applications during mid to late summer when higher temperatures may suppress appressorial formation.

In a previous study of approssorial formation (Wang and Kerns, 2015), *C. cereale* inoculated ABG and CBG leaves under a controlled environment. Detached leaves were inoculated in spore suspension of the pathogen and then incubated in petri dishes with high relative humidity sitting in dark incubators set at 12 to 34°C. It suspected that disease development under the incubators was dissimilar with field conditions, as the artificial environment did not simulate the real environment in the field. *Colletotrichum cereale* may produce appressoria rapidly once the daytime temperature reaches a desired temperature of appressorial formation, even if the average temperature of the particular day is low. Additionally, we assumed that using three consecutive days could simulate the accumulated hours for appressorial formation. However, it was possible that the accumulated hour of a particular temperature already resulted a high rate of appressorial formation before the daily average temperature reached three consecutive days threshold of the temperature, since the temperature fluctuated in consecutive days. Wang and Kerns (2015) already demonstrated that appresorial formation rate of *C. cereale* reached 90% within 48 hours PI under 12°C environment, and within 24 hours PI under 18 to 28°C environment. In our study, there were approximately 63 hours of temperatures between 10 and 14°C and 35 hours between 14 and 18°C from 1 through 14 Apr. 2014 (Data not shown). There were approximately 25 hours between 18 and 22°C from 11 to 14 Apr. 2014. Those accumulated hours could be enough for most of the conidia in the field producing appressoria, although the 10°C threshold occurred on 14 Apr. 2014. Therefore, the three consecutive days method failed to accurately identify the timing where the appressorial formation would have occurred and peaked before the threshold was reached.

On the other hand, the disease cycle of *C. cereale* is speculated to be polycyclic, which means it can produce multiple inoculum by conidia in one year (Agrios, 2005). When chlorothalonil was applied, no matter primary and secondary infection of *C. cereale* was inhibited. During 2014, anthracnose severity of 10°C, 14°C, 18°C and conventional treatments was kept almost zero until the final application. On 10 days after treatments (DAT), increased severity of these treatments was observed. We assumed that increased disease severity would be caused by the resumed secondary inoculum, regardless of the initial timing of fungicide. The severity of 22°C was reduced compared to untreated control (UTC) when chlorothalonil application initialed. During 2015, since the final treatment of 10°C, 14°C, 18°C and conventional treatments was 5 weeks earlier than 2014, the secondary infection resumed earlier than 2014. Thus, anthracnose severity of those treatments was 10 to 18% higher than 2014 in the beginning of July, due to the continued infection after the final treatment. In July 2015, daily temperature was around the optimum temperature of *C. cereale* appressorial formation, within could result that secondary inoculum of the pathogen occurred rapidly. Without the suppression of fungicide, disease developed strongly in plots of 10°C, 14°C, 18°C and conventional treatments regardless of the initial timing. The fungicide worked well in the 22°C treatment, as it reduced disease severity compared to all other treatments on 3 to 16 DAT.

CONCLUSION

The method to estimate fungicide application timing based on temperature threshold did not reduce anthracnose severity compared to conventional treatment. Once the fungicide application stopped, the disease severity of 10°C, 14°C and 18°C treatments increased to a similar level as the conventional treatment. It is speculated that predicting initial fungicide

application timing using average air temperature of three consecutive days is not appropriate under field condition compared to the controlled environment study. An alternative experiment design would be needed to predict the timing of the initial application. On the other hand, even if initial infection was reduced, there is wide enough temperature range throughout the Northeast for the pathogen to infect the host and produce secondary inoculum which may reduce the relvance of early applications targeting primary infection.

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Figure 1. Average daily air temperature at the Plant Science Research and Education in Storrs, CT during 2014 and 2015.

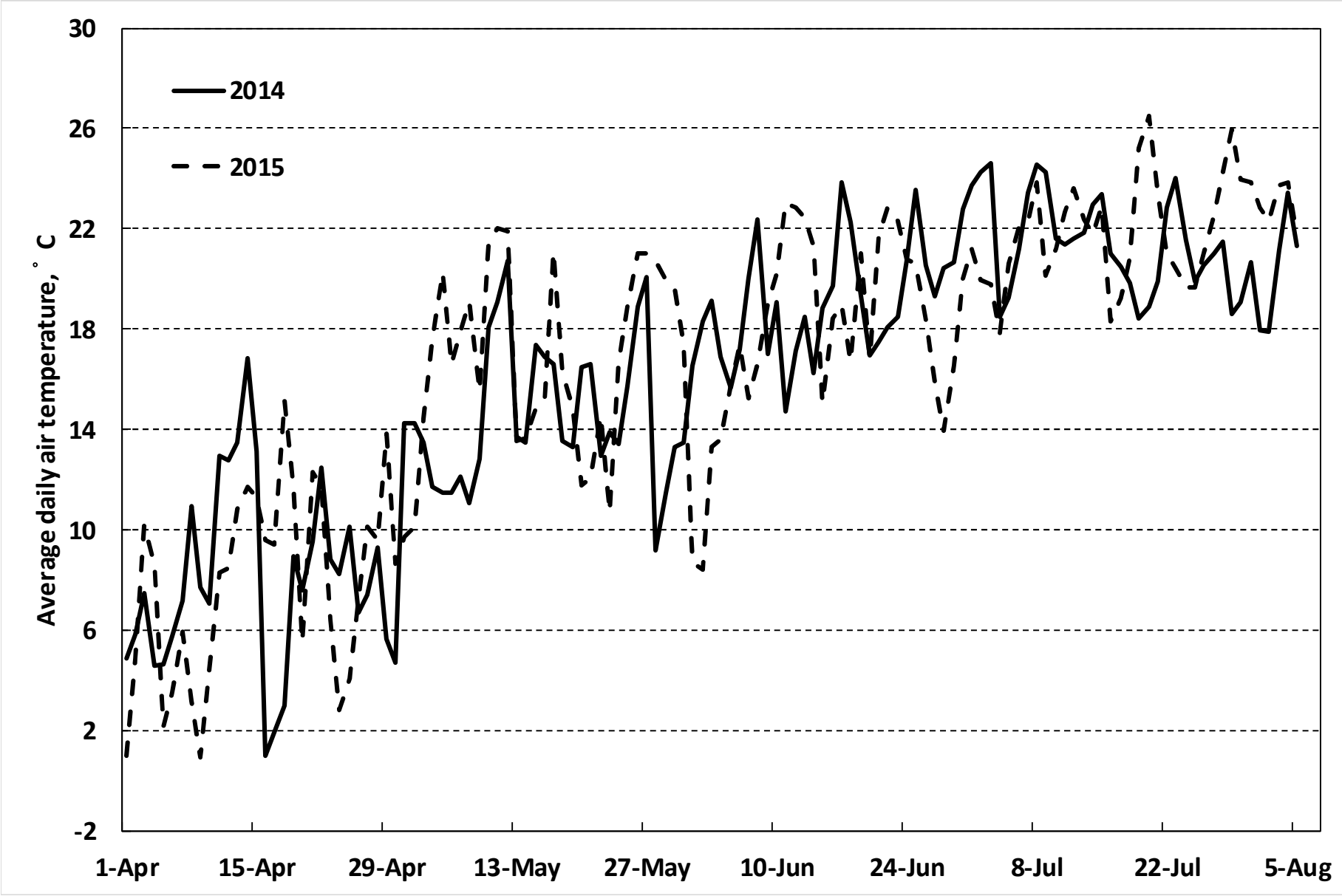


Table 1. Application dates of fungicide timing treatments for anthracnose control based on temperature threshold in Storrs, CT during 2014 and 2015.

	Application Dates													
2014	14 Apr	21 Apr	28 Apr	5 May	12 May	19 May	26 May	2 June	9 June	16 June	26 June	1 July	2 July	11 July
Timing	---------- Number of Applications ----------													
10°C	1	2	3	4	5	6	7	8	9	10	11	12		
14°C					1	2	3	4	5	6	7	8		
18°C					1	2	3	4	5	6	7	8		
22°C													1	2
Conv†								1	2	3	4	5		
2015	4 May	5 May	11 May	12 May	18 May	19 May	25 May	26 May	13 July	20 July				
Timing	---------- Number of Applications ----------													
10°C	1		2		3		4							
14°C		1		2		3		4						
18°C				1		2		3						
22°C									1	2				
Conv					1		2							

†Conventional timing.

Table 2. Analysis of variance for anthracnose severity affected by initial application timing based on air temperature threshold for annual bluegrass putting green turf in Storrs, CT during 2014 and 2015.

	Days After Final Treatment		Source of Variation
Evaluation Date	10°C, 14°C, 18°C and CONV[†]	22°C[‡]	Temperature Threshold[¶]
2014	---------- Number of applications --------		--- Significant levels ---
2 July	1		***
11 July	10	0	***
18 July	17	7	***
28 July	27	17	***
4 Aug	34	24	***
AUDPC[§]	--	--	***
2015			
7 July	43		NS
16 July	52		***
23 July	59	3	*
29 July	65	9	**
5 Aug	72	16	***
AUDPC	--	--	**

*, **, *** Significant at the 0.05, 0.01, 0.001 probability level, respectively; NS Not significant at p>0.05.

[†] Application dates for 10°C treatment are 14, 21, 28 Apr, 5, 12, 19, 26 May, 2, 9, 16, 26 Jun and 1Jul in 2014 and 4, 11, 18, 25 May in 2015. Application dates for 14°C treatment are 12, 19, 26 May, 2, 9, 16, 26 Jun and 1Jul in 2014 and 5, 12, 19 and 26 May in 2015. Application dates for 18°C treatment are 12, 19, 26 May, 2, 9, 16, 26 Jun and 1Jul in 2014 and 12, 19, and 26 May in 2015. Application dates for conventional timing treatment are 2, 9, 16, 26 Jun and 1Jul in 2014 and 18, 25 May in 2015. All treatments were sprayed Chlorothalonil at rate of 8 kg a.i. ha^{-1}.

[‡] Application dates for 22°C treatment are 2 Jul, 11 Jul in 2014 and 13 Jul, 20 Jul in 2015.

[§] Area under the disease progress curve.

[¶] Initial fungicide applications were made when the average daily air temperature reached 10°C, 14°C, 18°C and 22°C for 3 consecutive days. A conventional preventing timing application was also made on 2 June 2014 and 18 May 2015.

Figure 2. Anthracnose severity influenced by initial fungicide application timing based on threshold air temperatures on annual bluegrass putting green turf in Storrs, CT during 2014. Chlorothalonil was applied at 8 kg a.i. ha^{-1}on 14, 21, 28 Apr, 5, 12, 19, 26 May, 2, 9, 16, 26 June and 1July, 5, 12, 19, 26 May, 2, 9, 16, 26 June and 1July, 5, 12, 19, 26 May, 2, 9, 16, 26 June and 1July, 2 July and 11 July, 2, 9, 16, 26 Jun and 1Jul for 10°C, 14°C, 18°C, 22°C and conventional fungicide timing, respectively. Area under the disease progress curve (AUDPC) values was separated and lettered using Fisher's LSD (p=0.05), where LSD = 197. Error Bars represent LSD equaling to 2.1, 7.4, 9.2, 9.5 and 7.2 on 2, 11, 18, 28 Jul and 4 Aug, respectively. † Dates after final treatment for 10°C, 14°C, 18°C and conventional fungicide timing. ‡ Dates after final treatment for 22°C treatment.

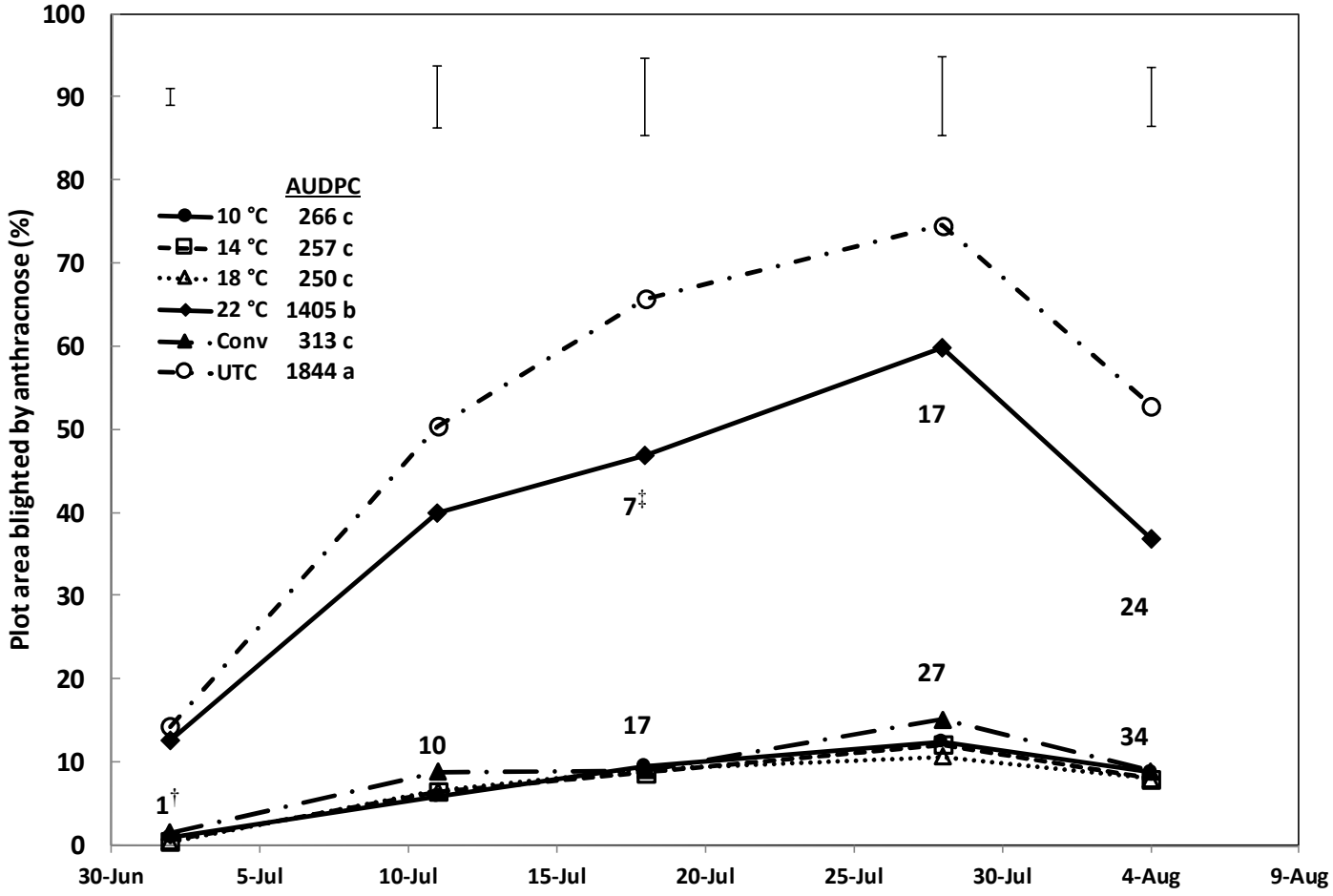
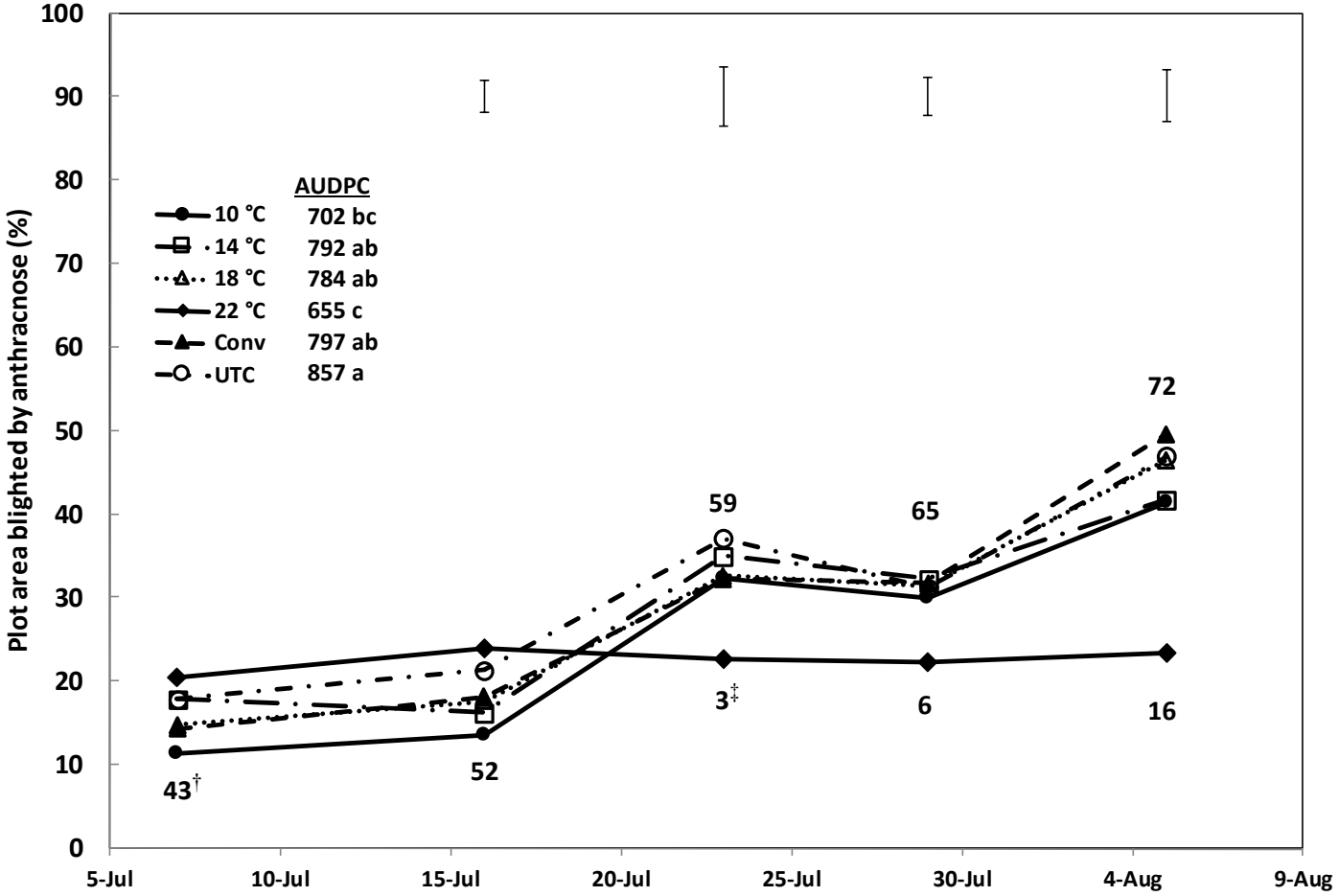


Figure 3. Anthracnose severity influenced by initial fungicide application timing based on threshold air temperatures on annual bluegrass putting green turf in Storrs, CT during 2015. Chlorothalonil was applied at 8 kg a.i. ha^{-1}on 4, 11, 18 and 25 May, 5, 12, 19 and 26 May, 12, 19 and 26 May, 13 July and 20 July, 18 May and 25 May for 10°C, 14°C, 18°C, 22°C and conventional fungicide timing, respectively. Area under the disease progress curve (AUDPC) values was separated and lettered using Fisher's LSD (p=0.05), where LSD = 105. Error Bars represent LSD equaling to 3.8, 7.1, 4.6 and 7.2 on 16, 23, 29 Jul and 5 Aug, respectively. † Dates after final treatment for 10°C, 14°C, 18°C and conventional fungicide timing. ‡ Dates after final treatment for 22°C treatment.



CHAPTER 2. Effect of Seasonal Nitrogen Fertilization and Plant Growth Regulators on Anthracnose Severity of Annual Bluegrass Putting Green Turf

ABSTRACT

Anthracnose (caused by *Colletotrichum cereale* sensu lato Crouch, Clarke, and Hillman) is a detrimental disease of annual bluegrass (ABG; *Poa annua* L.) putting greens. Ethephon and trinexapac-ethyl applied together for seedhead and vegetative control can reduce anthracnose severity, although this effect has been inconsistent in previous research. Moderate nitrogen (N) fertilization can improve ABG tolerance to anthracnose. However, the influence of seasonal N allocation on the ability of plant growth regulators to reduce anthracnose is not well understood. A two-year field study was established in late 2013 to evaluate potential interactions between seasonal N fertilization programs, ethephon (ET), and trinexapac-ethyl (TE) application interval on anthracnose severity of ABG putting green turf. Nitrogen treatments included spring or fall applications of 48.8 kg ha^{-1}, or a split application of 12.2 and 36.6 kg ha^{-1} applied spring and fall respectively. Ethephon was applied at 0 or 3.8 kg a.i. ha^{-1} twice in April. Trinexapac-ethyl treatment intervals consisted of none, 14 d interval, or every 200 growing degree days (GDD) base 0°C from mid-April through early September, applied at 0.05 kg a.i. ha^{-1}. Surprisingly, no consistent interaction of seasonal N allocation timing and plant growth regulators was observed. However, these factors influenced anthracnose severity independently. Spring N treatments consistently reduced disease severity compared to fall-only treatments from late June through early August 2014. Ethephon treated turf consistently had reduced anthracnose severity throughout 2014 and had no consistent effect in 2015. Trinexapac-ethyl consistently reduced anthracnose severity regardless of application interval in both years. However, TE applied every 200 GDD reduced disease severity more than TE every 14 d during July and August. Results to

date suggest spring rather than fall N fertilization and TE applied based on growing degree day (GDD) model can reduce anthracnose on annual bluegrass putting green turf.

INTRODUCTION

Anthracnose (caused by *Colletotrichum cereale* sensu lato Crouch, Clarke, and Hillman; Crouch et al., 2006) is a serious disease of cool-season (e.g., *Poa annua* L. and *Agrostis* spp.) putting green turf. Initial disease, symptoms consist of small, bronze-yellow spots and large irregularly shaped areas of blighted turf on putting greens (Smiley et al., 2005; Vargas, 2005). On individual plants, stem and leaf tissues are often chlorotic, turning necrotic and crowns may exhibit a dark, water-soaked rot. Signs of disease include saucer- or cushion-shaped fruiting bodies, called acervuli found on infected plant tissue (Smiley et al., 2005). Melanized, hair-like setae are unique structures, produced within acervuli, used to identify the pathogen. *Colletotrichum cereale* also produces abundant single-celled, hyaline, crescent-shaped conidia within acervuli.

Cultural practices including N fertilization, mowing, rolling, plant growth regulators, verticutting, topdressing and irrigation are known to influence anthracnose severity of annual bluegrass (ABG) turf (Inguagiato et al., 2008; Inguagiato et al., 2009b; Inguagiato et al., 2012; Roberts and Murphy, 2014). Nitrogen fertility is one of the most influential cultural practices with insufficient levels enhancing anthracnose severity (Inguagiato et al., 2008). Annual N fertility for general health and playability of ABG putting greens, has been recommended at 195 to 293 kg ha^{-1} per year (Vargas and Turgeon, 2004). Inguagiato et al. (2008) demonstrated annual N fertility at 176.9 to 178.3 kg ha^{-1} reduced disease severity 5 to 24% compared to annual N application totaling 90.3 to 93.8 kg ha^{-1} on ABG putting green. Whereas, an annual N

application at 146 kg ha^{-1} reduced anthracnose severity on an ABG fairway turf compared to 292 kg ha^{-1} annual N application (Danneberger et al., 1983). These studies demonstrate that moderate annual N fertilization generally reduces anthracnose severity compared to higher or lower rates of N fertilization.

However, few studies have evaluated the impact of N application timing with respect to seasonal allocation on anthracnose severity of ABG putting greens. Typically, N is applied to ABG putting greens as frequent, low-rate applications (e.g., 4.9 kg ha^{-1} every 7 to 14 d) from spring to early fall; followed by a high rate (e.g., 48.8 kg ha^{-1}) application in October to November (Vargas, 2005). Danneberger et al. (1983) conducted a study evaluating seasonal N fertility programs on ABG fairway turf that emphasized higher rate April and May applications with moderate summer applications through September, except during July; compared to moderate summer applications from June through September with a high rate November application. Results indicated that N applications emphasizing summer + fall more effectively reduced anthracnose during July than applications during spring + summer months, excluding July. They speculated that continuous fertility during summer months when ABG roots were declining due to environmental stress, was important to maintaining nutritional status and thus, resistance to the pathogen. Whereas, the spring + summer program, that specifically omitted July applications, likely resulted in nutrient deficiencies during the time when plants were most susceptible to anthracnose. This study concluded that summer N fertilization was important to reducing anthracnose severity. However, the contribution of moderate spring and fall N applications on the ability of light-frequent summer applications to suppress anthracnose were not clearly evaluated in this earlier study. The effect of seasonal allocation of N on anthracnose of ABG turf is still limited.

Plant growth regulators (e.g., ethephon and trinexapac-ethyl) are routinely applied to golf course putting greens to prevent seedhead formation and reduce clipping yields. Ethephon (ET; [(2-Chloroethyl) phosphonic acid]) is used as a seedhead suppressant that releases ethylene in plant tissue (Foster et al., 1992). Ethephon has been demonstrated to inhibit seedhead production in ABG turf (Eggens et al., 1989; Haguewood et al., 2013).

Trinexapac-ethyl (TE; [4-(cyclopropyl-α-hydroxy-methylene)-3,5-dioxocyclohexanecarboxylic acid ethylester]) is a widely used plant growth regulator, which interferes with gibberellic acid (GA) production late in the biosynthesis pathway. Trinexapac-ethyl inhibits hydroxylation of GA_{20} to GA_1 by blocking the activity of regulatory enzyme 3β-hydroxylase (Adams et al. 1992; Hedden 1991; Rademacher, 2000). It has been demonstrated that application of TE on perennial ryegrass (*Lolium perenne* L.) can reduce leaf elongation rate and improve tiller density (Ervin and Koski, 1998). Clipping yields of bermudagrass (*Cynodon dactylon* × *C. transvaalensis* Burtt-Davy), creeping bentgrass (*Agrostis stolonifera* L.;CBG), and Kentucky bluegrass (*Poa pratensis* L.) have been shown to be reduced by TE (Lickfeldt et al., 2001; McCullough et al. 2007). It also reported that TE can improve turf quality on both cool- and warm-season turfgrass (Inguagiato et al., 2008; Lickfeldt et al., 2001; McCullough et al., 2006).

Prohexadione-Ca (calcium;1-(4-carboxy-2,6-dioxocyclohexylidene)propan-1-olate; PC) is another growth regulator, recently registered for turfgrass in 2015, that also inhibits GA biosynthesis late in the pathway (Rademacher, 2000). Previous studies showed that PC reduced vegetative growth of cherry (*Prunus avium* L.), peanut (*Arachis hypogaea* L.), apple (*Malus domestica* Borkh.) and turfgrasses, including Kentucky bluegrass, perennial ryegrass, bermudagrass and zoysiagrass (*Zoysia japonica* Steud.; Beam, 2004; Elfving et al., 2003;

Mitchem et al., 1996; Unrath, 1999). Obear and Kreuser (2014) demonstrated that the effect duration of PC on CBG putting green was approximately 50% longer than TE, although it suppressed CBG growth at a similar level compared to TE.

Plant growth regulators have been shown to reduce anthracnose severity (Inguagiato et al., 2008; Inguagiato et al., 2010). However, the effects of these products in controlled field studies have not always been consistent across years, and in some cases disease reductions have been dependent on combinations of seedhead suppression with season-long vegetative growth regulation. The effect of ET on anthracnose was reported in 2010 (Inguagiato et al.) from a study on an ABG putting green turf. Two applications of ET at 3.8 kg a.i. ha^{-1}, made prior to seedhead emergence, reduced anthracnose 3 to 22% compared to no ET treatment in two out of the three years of the study.

Trinexapac-ethyl can also reduce anthracnose severity of ABG putting green turf. However, the reduction associated with TE was again, variable among years in previous studies (Inguagiato et al., 2008; 2009a; 2010). Trinexapac-ethyl applied alone at 0.05 kg a.i. ha^{-1} reduced anthracnose severity 7 to 13% during the first two years of a 3-yr study on ABG putting green turf (Inguagiato et al., 2009a). The effect of the PGR on anthracnose during the third year was dependent on prior application of mefluidide, a PGR used to suppress seedhead production. The effect of TE, in the absence of mefluidide was a slight increase in disease during June and July. Studies evaluating various TE application rates and intervals also identified inconsistent effects between years. During one of three years, anthracnose declined linearly with increasing rate (none, 0.04, 0.05, and 0.08 kg a.i. ha^{-1}), and shorter intervals (7 vs. 14d) reduced disease (Inguagiato et al., 2009a). However, TE had no effect on disease during the remainder of the study regardless of rate or interval.

The effect of TE on anthracnose can be dependent on previous application of a seedhead inhibitor (Inguagiato et al., 2009a). The combination of mefluidide applied during the spring for seedhead suppression, followed by summer long applications of TE reduced disease 6 to 14% compared to turf receiving only one of these PGRs. The addition of frequent N applications enhanced the effect of ME + TE combinations, reducing anthracnose severity ~22% compared to similarly fertilized turf treated with ME or TE alone.

These studies demonstrate that PGRs can help suppress anthracnose severity, however their effects are inconsistent. Authors of these studies noted that disease reductions observed in TE and ET treated turf occurred during years when increased rates of N were applied to the field to encourage recovery incurred in the study area the previous year. They speculated that increased spring N coinciding with initiation of PGR programs may be required to optimize the beneficial effects of PGRs on anthracnose. However, the hypothesis was never tested. Additional speculation was noted by Inguagiato et al. (2010) that inconsistent TE effects, particularly during high temperatures may be due to increased metabolism of the active ingredient, thus resulting in a post-inhibition growth enhancement (PIGE) that consumes carbohydrates during periods of heat and drought stress, predisposing turf to anthracnose (Inguagiato, et al. 2009a). Beasley and Branham (2005) demonstrated that TE activity in Kentucky bluegrass was directly related to air temperature, and the metabolism rate was doubled by doubled air temperature. Recently, a growing degree day (GDD) model has been developed to optimize TE reapplication timing to avoid (PIGE) associated with high temperatures (Kreuser and Soldat, 2011). However, it is unknown whether this GDD based model could improve the consistency of TE to suppress anthracnose.

Plant growth regulators have been demonstrated to have potential as cultural practices used to manage anthracnose in ABG putting green turf. However, the consistency of the effect, and extent of disease suppression have been variable among years in previous studies. Reasons for this apparent inconsistency are not well understood, although the influence of seasonal N fertility in combination with PGRs and environmental impacts on trinexapac-ethyl metabolism have been implicated. Identifying how PGRs interact with increased spring N fertility and use of recently developed GDD models for TE re-application may improve the efficacy of PGRs in an anthracnose management program. Therefore, the objectives of this study were to evaluate i) potential interactions between seasonal nitrogen fertilization programs, ethephon, and trinexapac-ethyl, ii) compare efficacy of GDD model based and calendar based TE application intervals, and prohexadione-Ca on anthracnose severity of ABG putting green turf.

MATERIALS AND METHODS

Site Description

The two-year field study was initiated in fall 2013 on a 4-yr old ABG putting green turf grown on a Paxton fine sandy loam (coarse-loamy, mixed, active, mesic Oxyaquic Dystrudepts) with a pH of 6.2 at the University of Connecticut Plant Science Research and Education Facility in Storrs, CT. The field was established on a previously existing mixed creeping bentgrass and ABG putting green derived from ABG sods and soil cores from golf courses throughout CT. Prior to initiating the study, creeping bentgrass was eliminated with fluazifop-P-butyl {Butyl (R)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoateat} 0.05 kg a.i. ha^{-1} on 16 May 2009. The field was core cultivated and additional soil cores from ABG putting greens obtained from Wethersfield Country Club in Wethersfield, CT were topdressed uniformly across

the field. All cores were pulverized with a verticutter and re-distributed with a box-link drag. Creeping bentgrass was eliminated from the site with fluazifop-P-butyl (Butyl (R)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoateat) 0.05 kg a.i. ha^{-1} on 16 May 2009. A monostand of ABG maintained at 3.2 mm was established by June 2010. Anthracnose developed uniformly throughout the study area each year from a naturally occurring infestation of *C. cereale*.

General Maintenance

Nitrogen (water soluble urea) was sprayed to the field uniformly to sustain turf vigor and encourage recovery from disease damage suffered by the previous year. For N and plant growth regulator trail, spring and fall N applications were considered as experiment treatments. During summer 2014 and 2015, N was sprayed approximately every 14 d at 4.9 kg ha^{-1}.

In trinexapac-ethyl and prohexadione-ca interval study, annual N was applied at 151.3 kg ha^{-1} in 2015. Spring and fall N were applied at 53.7 and 36.6 kg ha^{-1}, respectively. During summer 2015, 4.9 kg N ha^{-1} were applied every 7 d from June through August.

The field was mowed 5 days wk^{-1} at a bench setting height of 3.2 mm with a walk mower (Jacobsen PGM 22). Sand topresssing was applied at approximately 0.2 L m^{-2} and incorporated with a coco-mat drag every 14-d from May to September each year. Irrigation was applied to the field as needed to avoid drought stress. Dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett) was preventively controlled in the study area from May through August in 2014 and June through August in 2015 with a rotation of vinclozolin [3-(3, 5-dichlorophenyl)-5-ethenyl-5-methyl-2, 4-oxazolidinedione] at 1.5 kg a.i. ha^{-1}, boscalid {3-pyridinecarboxamide, 2-chloro-N-[4'-chloro(1,1'-biphenyl)-2-yl]} at 0.4 kg a.i. ha^{-1} and fluazinam {[3-chloro-N-[3-chloro-2,6-dinitro-4-trifluoromethyl)phenyl]-5-trifluoromethyl-2-pyridinamine]} at 0.6 kg a.i. ha^{-1} every 14 d.

Flutolanil {N-[3-(1-methylethoxy) phenyl]-2-[trifluoromethyl] benzamide} at 3.2 to 6.4 kg a.i. ha^{-1} during June and July 2014 and fluazinam {[3-chloro-N-[3-chloro-2,6-dinitro-4-trifluoromethyl)phenyl]-5-trifluoromethyl-2-pyridinamine]} at 0.6 kg a.i. ha^{-1} in July 2015 were applied to control brown patch (*Rhizoctonia solani* Kuhn). Algae was suppressed by zinc ion and manganese ethylenebisdithiocarbamate {[2-(dithiocarboxyamino)ethylamino]-sulfoniumylidenemethanethiolate; zinc; manganese}applied once $month^{-1}$ at 23.8 kg a.i. ha^{-1} from June to August 2014. Downy mildew (*Sclerophthora macrospora* (Sacc.) Thirum) and Pythium blight (*Pythium aphanidermatum* (Edson) Fitzp.) were controlled with mefenoxam {(R,S)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester}at 0.7 kg a.i. ha^{-1} on 29 Apr 2014 and 9 Jul 2015. The anthracnose epiphytotic was arrested between study years with applications of polyoxin D zinc salt { zinc 5-[[2-amino-5-O-(aminocarbonyl)-2-deoxy-Lxylonoyl]amino]-1-(5-carboxy-3,4-dihydro-2,4- dioxo-1(2H)-pyrimidinyl)-1,5-dideoxy-β-Dallofuranuronate} at 0.3 kg a.i. ha^{-1} and chlorothalonil (tetrachloroisophthalonitrile) at 8 kg a.i. ha^{-1} to promote turf recovery. Annual bluegrass weevil [*Listronotus maculicollis* (Kirby)] adults and larvae were eliminated respectively with lambda-cyhalothrin [1α(S), 3α(Z)]-(±)-cyano(3-phenoxybenzyl)methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2,-dimethylcyclopropanecarboxylate} at 0.07 kg a.i. ha^{-1}, Dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate at 9.1 kg a.i. ha^{-1} in May 2014 and Chlorantraniliprole {5-bromo-N-[4-chloro-2-methyl-6-(methylcarbamoyl)phenyl]-2-(3-chloropyridin-2-yl)pyrazole-3-carboxamide} at 0.2 kg a.i. ha^{-1} in June 2015.

Treatment and Experimental Design

Seasonal Nitrogen and Plant Growth Regulator Study

During 2013-2014, the study was conducted as a 3 × 2 × 3 factorial randomized complete block design with four replications. Seasonal N programs included spring or fall applications of 48.8 kg ha^{-1}, or a split application of 12.2 and 36.6 kg ha^{-1} applied spring and fall, respectively. Nitrogen was applied uniformly to all plots at 4.9 kg ha^{-1} every 14 d from May to August. Fall N applications were applied on 15 October and 7 November 2013, and spring N applications were applied on 11 and 25 April 2014. Total annual N input of each treatment was 117.4 kg ha^{-1}. Ethephon was applied at 0 or 3.8 kg a.i. ha^{-1} twice on 10 and 25 April once 110 to 167 GDD base 0°C had accumulated. Trinexapac-ethyl intervals consisted of none, every 14-d, or every 200 GDD base 0°C from 10 April through end of August 2014, applied at 0.05 kg a.i. ha^{-1}. Trinexapac-ethyl of 200 GDD interval was applied on 10 April, 2, 15, 27 May, 9, 19, 29 June, 8, 17, 28 July, and 5, 14, 25 August.

Due to winterkill in 2014 and loss of plots, the experiment was reduced to a 2 × 2 × 2 factorial design during 2015. Seasonal N programs had spring application of 48.8 kg ha^{-1}, or a split application of 12.2 and 36.6 kg ha^{-1} applied spring and fall, respectively. Nitrogen was applied uniformly to all plots at 4.9 kg ha^{-1} as urea every 14 d from May to August. Fall application of the split N treatment was sprayed on 16 September and 2 October 2014. Spring applications were applied on 17 April and 1 May 2015. Maintenance N was applied approximately every 14 d at 4.9 kg ha^{-1} from May to August, with a 122 kg N ha^{-1} input totally. Ethephon was applied at 0 or 3.8 kg a.i. ha^{-1} twice on 17 April and 1 May. Trinexapac-ethyl was applied at 0 or 0.05 kg a.i. ha^{-1} every 14 d from 16 April through 10 September. All treatments

were applied using a hand-held CO_2 powered sprayer with a single AI9508E flat fan nozzle (TeeJet Technologies; Glendale Heights, IL) calibrated to deliver 814 L ha^{-1} at 276 kPa.

Trinexapac-ethyl and Prohexadione-Ca Interval Study

The study consisted of five treatments arranged in a randomized complete block design with four replications. Treatments included an untreated control, TE applied every 14 d or 200 GDD at 0.05 kg a.i. ha^{-1}, and PC applied every 14 d or 300 GDD at 0.08 kg a.i. ha^{-1}. Plant growth regulator applications of calendar interval were made eight times from 4 June through 10 September 2015. Trinexapac-ethyl of 200 GDD interval was applied on 4, 15, 26 June; 3, 14 20, 30 July; 7, 17, 26 August; and 11, 12 September. Prohexadione-Ca of 300 GDD interval was applied on 4,18 June; 3,19 July; 3,17 August; and 1 September. All treatments were applied using a hand-held CO_2 pressurized sprayer with a single AI9508E flat fan nozzle (TeeJet Technologies; Glendale Heights, IL) calibrated to deliver 814 L ha^{-1} at 276 kPa.

Data Collection and Analysis

Anthracnose severity was assessed as the percent plot area blighted by *C. cereale* using a line intercept grid count method described by Inguagiato et al. (2008). For the N-PGR study, data were recorded every 7 d from 23 June through 5 Sep. 2014, and 5 June through 12 Sep. 2015. For PGR study, data were collected every 7 d from 6 July through early 16 September 2015. Turf quality was visually rated on a 1 to 9 scale (where 9 represented the best quality and 6 the minimum acceptable level) in the same period and interval. Turf density, uniformity, color, phytotoxicity, seedhead expression and disease severity were considered into turf quality rating. Seedhead expression assessment of N-PGR study was visually estimated as percent plot area containing seedheads in May and June each year. In the N-PGR study, clippings were collected

by mowing one 1.27-m pass down the center of each plot approximately 14 d from 7 May through 27 August, 20 October 2014, and 18 May through 27 August 2015. In the PGR study clippings were collected from 18 June through 27 August 2015. Area of clipping collection were approximately 1.4 and 0.7 m^2 in 2014 and 2015, respectively. Clippings were sent into 60 to 70°C drying room for three days, seedheads,weeds, and debriswas removed by 1-mm sieve, and then remaining tissue was weighed. Clipping N concentrations were quantified by nitrogen analyzer (TruMac®, LECO Corporation, Joseph, MI). All data were subjected to analysis of variance using MIXED procedure in the Statistical Analysis System software v. 9.4 (SAS Institute Inc., Cary, NC). Treatment means were separated using Fisher's protected least significant difference test (p=0.05).

RESULTS

Seasonal Nitrogen and Plant Growth Regulator Study

Anthracnose Severity

Anthracnose developed from a natural infestation throughout the study area during late July 2014 and 2015. During 2014, the seasonal N main effect significantly affected anthracnose severity from 23 June through 6 Aug. (Table 1). Fall N application resulted in 2 to 8% higher anthracnose than spring and spring + fall applications throughout those dates. No difference was observed between the spring + fall N treatment and the spring N treatment on any observation dates throughout 2014. The area under the disease progress curve of fall N treatment was approximately 20% greater than the other two treatments (Fig. 1).

Ethephon generally reduced disease severity 3 to 15% compared to no ET treatment from 2 July through 14 Aug 2014 (Fig. 2). Area under the disease progress curve was 26% lower in ET-treated compared to non-ET treated turf (Fig 2).

In all observation dates of 2014, significant differences were observed among TE application intervals (Table 1). Trinexapac-ethyl applied every 14 d (TE14) reduced anthracnose 1 to 6% on 8 out of 11 observation dates compared to no TE treatment (Fig. 3). Similarly, TE applied every 200 GDD (TE200) also reduced anthracnose 1 to 14% compared to no TE treatment throughout 2014. However, TE200 had 7 and 5% less disease than TE14 on 30 July and 6 Aug., respectively. Significant differences were also observed among AUDPC values for TE treatments, with both TE14 and TE200 having 30% lower epidemic values, regardless of interval, than non-TE treated turf (Fig. 3).

Interactions involving seasonal nitrogen timing and PGRs were observed on only two of 11 observations during 2014 (Table 1). The first involving seasonal N and ET occurred as symptoms initially developed throughout the study on 23 June. Ethephon reduced anthracnose severity when applied to turf receiving the spring N allocation, but had no effect on turf emphasizing fall or spring + fall fertility (data not shown). Within ET treated turf, disease decreased incrementally among seasonal timings, as the bulk of N allocation shifted from fall to spring (data not shown). A separate interaction between seasonal N and TE interval was observed 6 Aug when disease pressure was high (Table 1). Generally, TE reduced anthracnose severity compared to non-TE treated turf, regardless of application interval, with no difference between TE14 and TE200 intervals. However, the effect of seasonal N was dependent on the application of TE. In the absence of TE, spring and spring + fall N timings reduced disease

compared to fall; whereas, wherever TE was applied, no differences were observed among seasonal N allocation programs (data not shown).

During 2015, no significant seasonal N main effect was observed, indicating similar as in 2014, that there was no difference between spring N applications and spring + fall N applications (Table 2; Fig. 4). Fall N applications were not evaluated in 2015 due to winterkill throughout the study area. On 5 of 12 assessment dates, significant differences were found between ET and non-ET treated turf, (Table 2). Ethephon reduced disease 7 and 8% compared to no ET treatment on 9 July and 7 Aug. 2015, respectively. However, ET increased 10 to 14% disease from 21 Aug. through 4 Sept. 2015. In addition, AUDPC for ET was not significantly different from non-ET treatment (Fig. 5). The trinexapac-ethyl main effect, significantly influenced anthracnose severity on 9 of 12 observation dates during the later half of the season (July – September) 2015 (Table 2). Trinexapac-ethyl applied every 14 d reduced disease severity 8 to 18% compared to no TE treatment. The TE GDD based interval was not evaluated in this study during 2015 due to a loss of turf because of winterkill. However, it was evaluated in a separate study described below. The AUDPC for TE14 was approximately 42% less than no TE treatment (Fig. 6).

No interactions influencing anthracnose severity were observed during 2015.

Turf Quality

During 2014, the seasonal N main effect had a significant effect on turf quality during May and June (Table 3). Both spring N programs improved turf quality compared to the fall N program during May and June 2014. Further quality improvement was observed in spring N programs compared to the split spring + fall program on 28 May. No differences in turf quality were observed among any seasonal N programs during July and August, when all plots received equivalent amounts of maintenance fertility.

Ethephon affected turf quality on 3 of 5 assessments during 2014 (Table 3). Plots treated with ET had greater turf quality on 28 May, 24 June, and 24 July compared to non-ET treated. An interaction between seasonal N and ET was observed during late-May and June, where the greatest turf quality was observed in spring applied N plots treated with ET (Table 4). However, specific effects of the interaction were variable between the two dates. In May, quality improved incrementally among N application timings as the allocation of N transitioned from fall to spring, regardless of ET. However, the effect of ET was dependent on N timing, where ET only improved quality in plots receiving spring N applications (Table 4). By June, ET improved quality regardless of N application timing. No differences were observed among N application timing in the absence of ET by this date; although spring N treatments improved quality of ET treated turf compared to fall applied N or applications split between spring + fall (Table 4).

Turf quality differences were also found among TE interval treatments on 4 of 5 observation dates. Trinexapac-ethyl applied every 14 d improved turf quality on only 2 of 5 observation dates compared to non-TE treated turf; whereas, the TE200 growing degree based applications improved quality on 4 of 5 dates during 2014. No interactions involving TE interval were detected.

During 2015, the spring N program improved turf quality compared to spring + fall N application on 7 May, only (Table 3). Ethephon had no effect on turf quality, until 4 September when ET decreased ABG quality compared to no ET plots (Table 3). Significant differences in turf quality between TE14 and no TE treatment were observed on 3 of 5 dates across both years (Table 3). The effect of TE applications every 14 d improved turf quality on observation dates during July, August and September. Growing degree day based TE applications were not

evaluated in this study during 2015, but are described in a separate study below. No interactions influencing turf quality were observed during 2015.

Seedhead Expression

During 2014, ABG seedheads were initially observed by early-May and subsided by late-June. A consistent interaction between seasonal N allocation and ET influenced seedhead expression on 3 of 4 observation dates during 2014 (Table 5). Results described focus on interaction means for seasonal N and ET rather than main effects on 19 May, 2 June, and 16 June. Ethephon always reduced seedhead expression 7 to 43% on each date regardless of seasonal N treatments (Table 6). However, the influence of seasonal N applications on seedhead expression was variable among dates and ET treatment. When ET was applied, the spring fertilization treatment initially had the fewest seedheads compared to split spring + fall or fall fertilization treatments on 19 May; and was similar on 2 June, although the split spring + fall treatment was statistically seperated, with no difference from fall or spring N applications. As seedheads waned by 16 June, no differences were observed among seasonal N treatments in the presence of ET. In the absence of ET, an inverse trend among seasonal N treatments was observed over the course of the seedhead-production window compared to when ET was applied. During peak seedhead expression (19 May) no differences were observed among seasonal N treatments; however, by 2 and 16 June 9 to 18% more seedheads were observed in the spring applied N treatment compared to split spring + fall or fall treatments (Table 6).

Trinexapac-ethyl, regardless of application interval, reduced seedheads approximately 1 and 10% compared to non-TE treated turf on 6 and 19 May, respectively.

During 2015, a seasonal N application by ET interaction was detected on one of 4 observation dates (Table 5). Results of the interaction on 3 June 2015 were similar to those

observed during 2014 as seedheads waned. Ethephon always reduced seedheads compared to non-ET treated turf; and spring N application increased seedheads compared to split spring + fall application without ET, but were no different when ET was applied (Table 6). Seasonal N did not have an effect on seedhead expression on any other date during 2015. However, the ET main effect reduced seedheads 8 to 49% on all other observation dates that year (Table 5). Trinexapac-ethyl had no effect on seedhead expression during 2015.

Clipping Yield

During 2014, seasonal N influenced clipping yield as a main effect or interaction on all but one observation date before disease severity increased above 10% plot area infested (Table 7). The spring-emphasis N program was comparable to the split spring + fall N program on the first harvest date (7 May), but increased clipping yield 21 to 25% compared to all N programs on the remaining dates during May and June, except 2 June. Similarly, an interaction with ET on 19 May, indicated the increase in clipping yield associated with spring N program compared to other N programs was evident only when ET was applied (Table 8).

Ethephon affected clipping yield on the first 4 of 5 harvest dates, however the effect differed during May and June (Table 7). During May, ET increased clipping yield 12 to 67% compared to non-ET treated turf. On 19 May, this effect was only apparent in plots receiving increased spring N fertility (Table 9). By June, ET had the opposite effect, reducing clipping yield 13 to 25% compared to non-ET treated plots, regardless of N or TE (Table 7).

The TE interval main effect influenced clipping yield on all harvest dates (Table 7). Trinexapac-ethyl applied based on the GDD model reduced clipping yield 24 to 36% compared to non-TE treated turf on all harvest dates, and 5 to 23% compared to TE14 on the first three

dates of the season (Table 7). Both TE intervals had fewer clippings than non-TE treated turf, but were no different from each other on the last two harvest dates in June.

During 2015, increased spring N applications had greater clipping yield compared to the split spring + fall program on 18 May (Table 8), and when applied in combination with TE on 1 June (Table 9). No seasonal N effect on clipping yield was apparent by mid-June. Ethephon had a similar effect on clipping yield as observed the previous year; where clippings were increased 57% in 18 May, but decreased 14% on 1 June. Surprisingly, TE had no effect on clipping yield during 2015, except influence it appeared to have on the seasonal N effect described above (Table 8).

Clipping Nitrogen Concentration

During 2014, all main effects intermittently affected clipping N concentration. The seasonal N main effect differences were apparent from 7 May through 30 June, and on 20 Oct. (6 of 10 evaluation dates) (Table 9). As expected, the spring emphasis N allocation and the split spring + fall programs had greater clipping N concentration compared to the fall emphasized program during May and June. Differences between spring and spring + fall programs were apparent during May, shortly after spring applications; however by June both programs contained similar N concentration. On 20 October, both programs involving fall N applications contained greater clipping N concentrations after fall nitrogen fertilization was applied.

There were significant differences in clipping N concentration between ET and no ET treatments on 7 out of 10 evaluation dates (Table 9). Generally, ET treated turf had greater clipping N concentration compared to non-ET treated on several observation dates from 19 May through 11 Aug. However, slightly lower N concentrations were detected in ET treated turf compared to non-ET treated on the first and last observation dates of 2014.

Trinexapac-ethyl occasionally increased clipping N concentration during 2014, with most effects occurring between May and July (Table 9). During May, TE increased N concentration compared to no TE treatment, regardless of application interval. Trinexapac-ethyl applied based on the GDD model continued to contain greater clipping N concentration compared to non-TE treated turf during early to mid-June and on 29 July. However, TE applied every 14-d did not differ from either non-TE treated or GDD based applications, on these later dates.

During 2015, few treatment effects were detected for clipping N concentration. A difference between spring and spring + fall programs was only observed on 18 May (Table 10). Clippings of spring N plots had higher N concentration than spring + fall N plots on that date. Ethephon resulted in a lower N concentration on 29 June, with no significant difference on remaining collection dates. Trinexapac-ethyl did not increase clipping N concentration until end of the summer in 2015. It resulted a slightly greater N concentration on 27 Aug. compared to no TE treatment.

Trinexapac-ethyl and Prohexadione-Ca Interval Study

Anthracnose Severity

Differences in anthracnose severity influenced by PGRs were observed on all evaluation dates except the first date (Table 12). Plant growth regulators averaged across application intervals consistently reduced anthracnose 3 to 21% compared to the untreated control from 22 July through 16 Sept 2015. Few differences (2 of 11 observation dates) in anthracnose severity were observed between TE and PC throughout the study. However, when differences where observed, TE treated turf had 6% less anthracnose than PC treated on 15 July when both were applied according to GDD; although no differences were observed on the same evaluation date when both were applied every 14-d. Conversely, PC had 7% less anthracnose than TE regardless

of application interval a month later on 12 Aug. Application of PGRs based on GDD models generally reduced (5 of 11 observations) anthracnose 6 to 8 % compared to the calendar-based 14 d application intervals, and reduced the severity of the epidemic (i.e., AUDPC) by 8%.

Turf Quality

Trinexapac-ethyl and PC improved turf quality compared to UTC, regardless of application interval, by 22 July as anthracnose severity was increasing throughout the study area (Table 12). Quality of turf among all PGR treatments remained greater than UTC through 19 August, although was no different from UTC during September. Application based on GDD improved turf quality compared to either PGR applied on a 14-d interval on 5 August, and 1 September. No turf quality differences between TE and PC were observed regardless of application interval.

Clipping Yield and Nitrogen Concentration

The effect of PGRs on clipping yield was most apparent on 18 June, shortly after the initial applications were made (Table 13). Prior to this date, TE14, PC14 and PC300 were applied once and TE200 was applied two times. The pooled effect of PGR type and application interval reduced clipping yield 22% compared to UTC on 18 June. Prohexadione-Ca reduced clipping yield greater than TE on this date, regardless of application interval. No differences were observed among any treatments on 29 June, although the pooled PGR effect again resulted in a 46% yield reduction compared to untreated on 13 July.

No differences in clipping N concentration were detected among any of the PGR treatments tested during this year.

DISCUSSION

Efficacy of ET and TE applied individually, or their interactive effects, to reduce anthracnose severity have been purported to be dependent on increased N application rates during spring months (Inguagiato et al., 2009a; Inguagiato et al., 2010). This study was conducted to determine whether increased spring N fertility is necessary for ET and TE to reduce anthracnose severity. Results from this study fail to support the hypothesis that ET and/or TE effects on anthracnose are exclusively dependent on spring N fertilization practices. These data suggest that each of these factors can independently reduce the disease, although increased spring N fertility did occasionally enhance anthracnose control associated with ET. However, as has been reported in previous studies these effects were variable between years.

Annual N fertilization programs that included a spring allocation of 6.1 to 24.4 kg ha^{-1} applied between mid- to late-April reduced anthracnose severity of ABG putting green turf compared to N programs emphasizing fall applications without spring applications. The beneficial effect of moderate N fertility applied during summer months on anthracnose is well documented (Inguagiato et al., 2008; Roberts et al., 2010; Inguagiato and Guillard, 2016). However, little has been published about the effect of spring and fall N fertilization practices on the disease. In this study, annual N programs emphasizing a spring allocation, or split spring + fall N allocations resulted in comparable levels of anthracnose during both years of the study. This suggests that even relatively small N contributions (i.e., 6.1 kg ha^{-1}) during spring appear to improve ABG tolerance to anthracnose. Nitrogen fertilization programs where at least 6.1 kg ha^{-1} of the annual total was applied during the spring (i.e., spring and spring + fall programs) reduced anthracnose compared to turf where fall fertility was emphasized. Similarly, turf quality was greater in both spring programs compared to the fall program. These effects were evident

during 2014, although were not evaluated during 2015 since winterkill reduced the available experimental plot area. However, this one-year result is consistent with an abstract of a two-year study by Schmid et al. (2011) which also observed that spring N fertility reduced anthracnose more than equivalent rates of N applied during fall.

Results from these two studies suggesting spring fertility is more effective in suppressing anthracnose and improving quality of ABG putting green turf contradict recommended fertilization practices for ABG and other cool-season turfgrasses (Beard, 1973; Vargas and Turgeon, 2004). Annual N fertility programs typically recommend the majority of N be applied from late-summer to fall, rather than spring. This is intended to avoid excessive foliar growth rather than root growth during spring, prior to summer stress (i.e., heat and drought). However, foliar N concentrations collected during 2014 were lowest during May and increased through July before reaching a plateau, regardless of N treatment. The increase in foliar N concentration observed among all treatments, from May to July is most likely due to mineralization in the soil resulting in plant available N. However, foliar N concentration was greater in both spring N programs compared to the fall program from May through mid-June. Recently, anthracnose severity has been modeled based on foliar N concentration; whereby the probability of anthracnose has been shown to increase at foliar N concentration $\leq$ to 33.5 g kg^{-1} (Inguagiato and Guillard, 2016). While N concentrations among all N programs remained below this reported critical threshold until June, both spring programs exceeded the threshold by 16 June, just before initial symptoms were observed; whereas the fall N program did not reach the critical threshold until 21 July. This delay in the fall N program to accumulate sufficient N, during spring, to meet the critical foliar N concentration may be related to the increased disease severity observed in

these plots during 2014. Conversely, supplemental spring N fertilization appears to contribute N to ABG plants during a time when N availability through mineralization is limiting.

Ethephon effects on anthracnose were highly variable between years in this study. This PGR consistently reduced anthracnose severity during 2014 and two observation dates in 2015, as disease was increasing. However, it later enhanced disease during the peak of the 2015 epidemic. As expected, ET reduced seedhead production during both years. Interestingly, similar to ET effects on anthracnose severity, its effect on foliar N concentration also varied between the two years of the study. In 2014, when ET consistently reduced anthracnose, foliar N concentrations were generally greater during summer months in ET treated turf compared to non-ET treated turf. Whereas during 2015, ET either had no effect, or reduced foliar N concentration compared to non-ET treated turf.

The inconsistency observed in this study among ET treatments between years has been previously reported in earlier studies evaluating the effect of ET on anthracnose severity (Inguagiato et al., 2010). Discussion regarding inconsistent effects in previous studies speculated that the ability of ET to suppress anthracnose may be dependent on increased spring N fertility. However, results from the current study examining the potential interaction between spring N and ET, fail to support this hypothesis. Very few interactions between ET and seasonal N fertility or TE were observed throughout the duration of this study. Several N × ET interactions were observed to influence anthracnose severity, turf quality and seedhead cover during 2014. These interactions generally showed less anthracnose, improved turf quality and fewer seedheads when a spring N program was applied to ET treated turf, although these interactions were infrequent, occurring on only one observation date for each dependent variable. Based on the lack of a consistent relationship between seasonal N fertility and ET within and

between years it is likely that some other factor may be responsible for the discrepancies in the effect of ET on anthracnose severity.

As previously stated, ET enhanced anthracnose severity on 3 observation dates during the peak of the 2015 epidemic. These data differ from those reported by Inguagiato et al. (2010). This previous study found ET either reduced anthracnose or had no effect, and concluded that despite the inconsistent effect on disease, seedhead suppression using ET should not increase the severity of anthracnose, or predispose turf to the disease. Results from this study, indicate that in some situations ET may intensify anthracnose epidemics. It is unclear as to why ET caused an increase in disease at this time, although it may be due in part to residual effects of severe winter stress that resulted in winterkill of portions of the experimental area. Increases in anthracnose severity observed among ET treated turf during 2015, suggests that turf practitioners may consider withholding ET applications during years following extreme winter conditions. However, further observations are necessary to validate this speculation.

Trinexapac-ethyl, regardless of application interval, frequently reduced anthracnose severity during both years of this study compared to non-TE treated turf. Few anthracnose differences between the GDD application model and calendar approach were observed for anthracnose severity during 2014. However, GDD based applications did provide further disease reductions compared to calendar-based timings (i.e., 14-d interval) on two dates during 2014 near the peak of the disease epidemic in late-July and early-August. The GDD-based application approach resulted in a total of 10 applications compared to 8 for the calendar approach by late-July when anthracnose differences were observed this year. In the companion study conducted during 2015, the GDD model reduced anthracnose compared to 14-d intervals ~50% of the evaluation dates.

These data support the hypothesis that use of GDD models for applying TE may result in a more consistent regulation of ABG growth, and reduce anthracnose severity. The GDD model was developed to estimate the rate of temperature-dependent TE metabolism in creeping bentgrass putting green turf (Kreuser and Soldat, 2011). As the plant metabolizes TE, the growth regulation effects dissipate. At increased summer temperatures TE may be metabolized to inactive levels in less than 14-d. This may result in a post-inhibition growth enhancement of foliage that could consume plant resources such as carbohydrates (Cooper et al., 1988; Spak et al., 1993; Kreuser and Soldat, 2011). This is particularly detrimental to turf during summer stress periods when respiration demands exceed photosynthetic production (Liu and Huang, 2001).

Trinexapac-ethyl applied using the GDD model has also been shown to reduce N losses 25 to 50% from creeping bentgrass putting green turf (Kreuser and Soldat, 2012). The previous study did not detect any effect of TE on clipping N content, however TE did reduce clipping yield, and therefore minimized N loss from turf as clippings. In our study, both TE application intervals reduced clipping yield, prior to disease, and also increased clipping N concentration compared to non-TE treated turf during May 2014. Further increases in clipping N concentration were occasionally observed in TE applied every 200 GDD on individual harvest dates during June and July compared to non-TE treated turf. The increase in N concentration in TE treated plots may be one way that TE helps ABG suppress anthracnose. However, despite reducing anthracnose severity during 2015, TE applied at either application interval had no effect on clipping yield or clipping N concentration. Thus, it appears that the effect of TE on anthracnose may involve factors other than clipping N concentration and clipping yield, that are considered in

this study. It is unknown why TE had little effect on these two variables during 2015, however it is possible that turf recovery due to severe winter stress may have influenced these results.

Results from this study indicate that annual N allocation timing (i.e., spring, spring + fall, fall), ET and TE application interval can independently influence anthracnose severity. While no consistent interactions between these factors were observed, increased spring N fertility in combination with ET or TE generally had beneficial effects on ABG putting green performance when they occurred. Nitrogen fertilization in the spring to supplement light-frequent summer applications is more effective at reducing anthracnose severity than fall applications. Ethephon can be used to reduce seedheads, and occasionally reduce anthracnose. However, turf practitioners may consider avoiding ET applications following severe winter conditions. The GDD model generally enhanced effects with TE resulting in significant differences from non-TE treated turf compared to calendar-based applications. Thus, use of a GDD model (200 GDD; base 0°C) for TE applications to ABG putting greens is a useful approach to improving consistency of anthracnose control.

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Table 1. Analysis of variance for anthracnose severity affected by seasonal nitrogen fertilization, ethephon and trinexapac-ethyl treatment interval for annual bluegrass putting green turf in Storrs, CT during 2014.

	Anthracnose Severity											
Source of variation	23 June	2 July	10 July	17 July	23 July	30 July	6 Aug	14 Aug	20 Aug	28 Aug	5 Sept	AUDPC[†]
	---------- Significant levels ----------											
Seasonal Nitrogen (N)[‡]	***	***	***	*	*	**	**	NS	NS	NS	NS	**
Ethephon (ET)[§]	NS	***	***	***	***	***	***	**	NS	NS	NS	***
Trinexapac-ethyl Interval (TE)[¶]	*	***	*	**	*	***	***	***	***	***	***	***
N x ET	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
N x TE	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS
ET x TE	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
N x ET x TE	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV, %	42.4	42.4	55.5	50.2	34.7	26.0	22.8	22.7	22.6	29.2	44.0	25.4

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively; NS Not significant at p>0.05.

[†] Area under the disease progress curve.

[‡] Nitrogen treatments were allocated seasonally emphasizing fall, spring + fall, and spring application timings. Fall N was applied as a split application of 24.4 kg ha^{-1} on 15 Oct. and 7 Nov. 2013, Spring + Fall N timings were applied as a split application of 18.3 kg ha^{-1} on 15 Oct. and 7 Nov. 2013 and a split application of 6.1 kg ha^{-1} on 11 and 25 Apr. 2014, and spring N was applied as a split application of 24.4 kg ha^{-1} on 11 and 25 Apr. 2014.

[§] Ethephon was applied as a split application of 3.8 kg a.i. ha^{-1} on 10 and 25 Apr. 2014.

[¶] Trinexapac-ethyl was applied every 14 d or every 200 GDD from 10 April to 27 August at 0.05 kg a.i. ha^{-1}.

Figure 1. Anthracnose severity influenced by seasonal nitrogen fertilization on annual bluegrass putting green turf in Storrs, CT during 2014. Nitrogen treatments were allocated seasonally emphasizing fall, spring + fall, and spring application timings. Fall N was applied as a split application of 24.4 kg ha^{-1} on 15 Oct. and 7 Nov. 2013, Spring + Fall N timings were applied as a split application of 18.3 kg ha^{-1} on 15 Oct. and 7 Nov. 2013 and a split application of 6.1 kg ha^{-1} on 11 and 25 Apr. 2014, and spring N was applied as a split application of 24.4 kg ha^{-1} on 11 and 25 Apr. 2014.

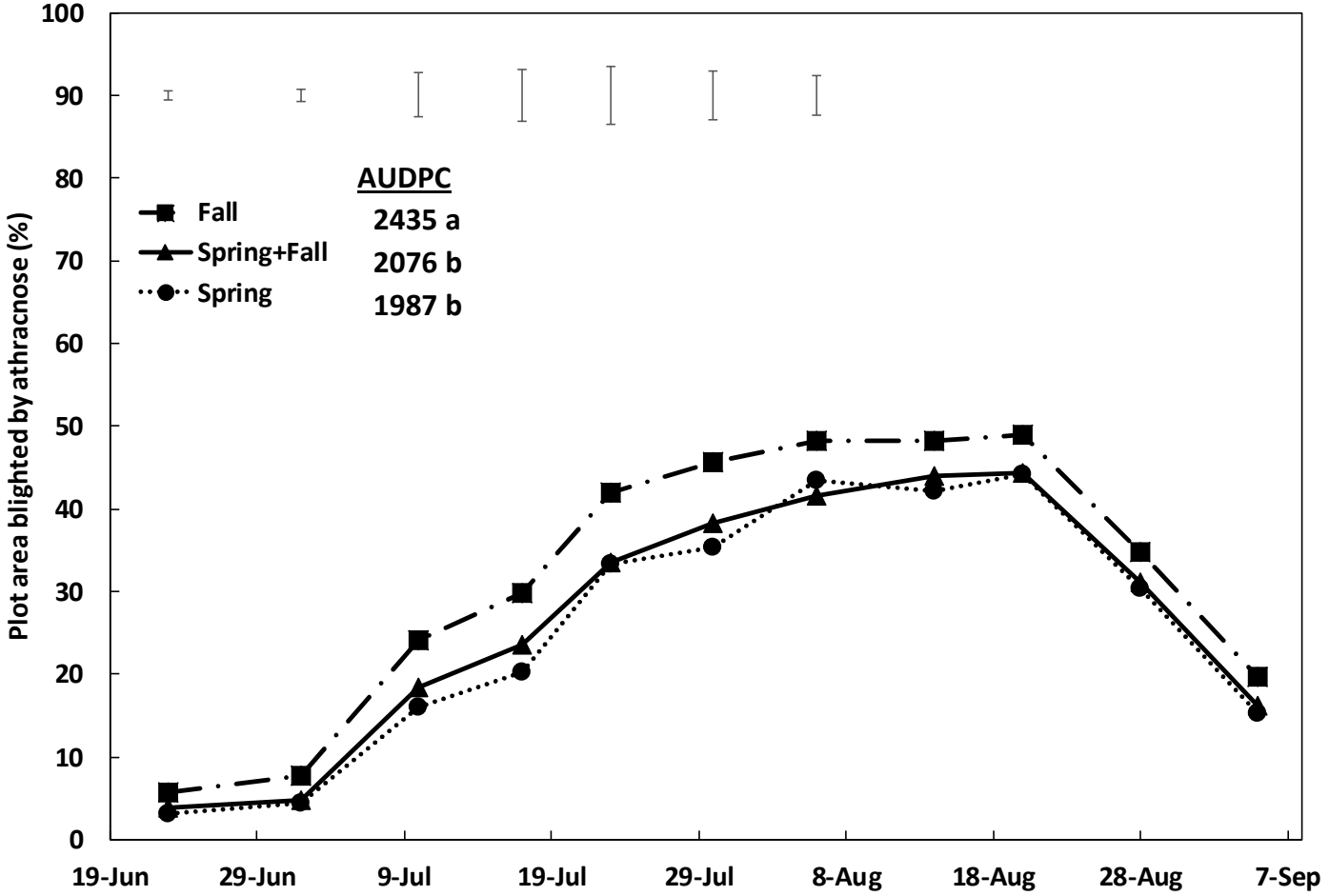

Figure 2. Anthracnose severity influenced by ethephon on annual bluegrass putting green turf in Storrs, CT during 2014. Ethephon was applied as a split application of 3.8 kg a.i. ha^{-1} on 10 and 25 Apr. 2014.

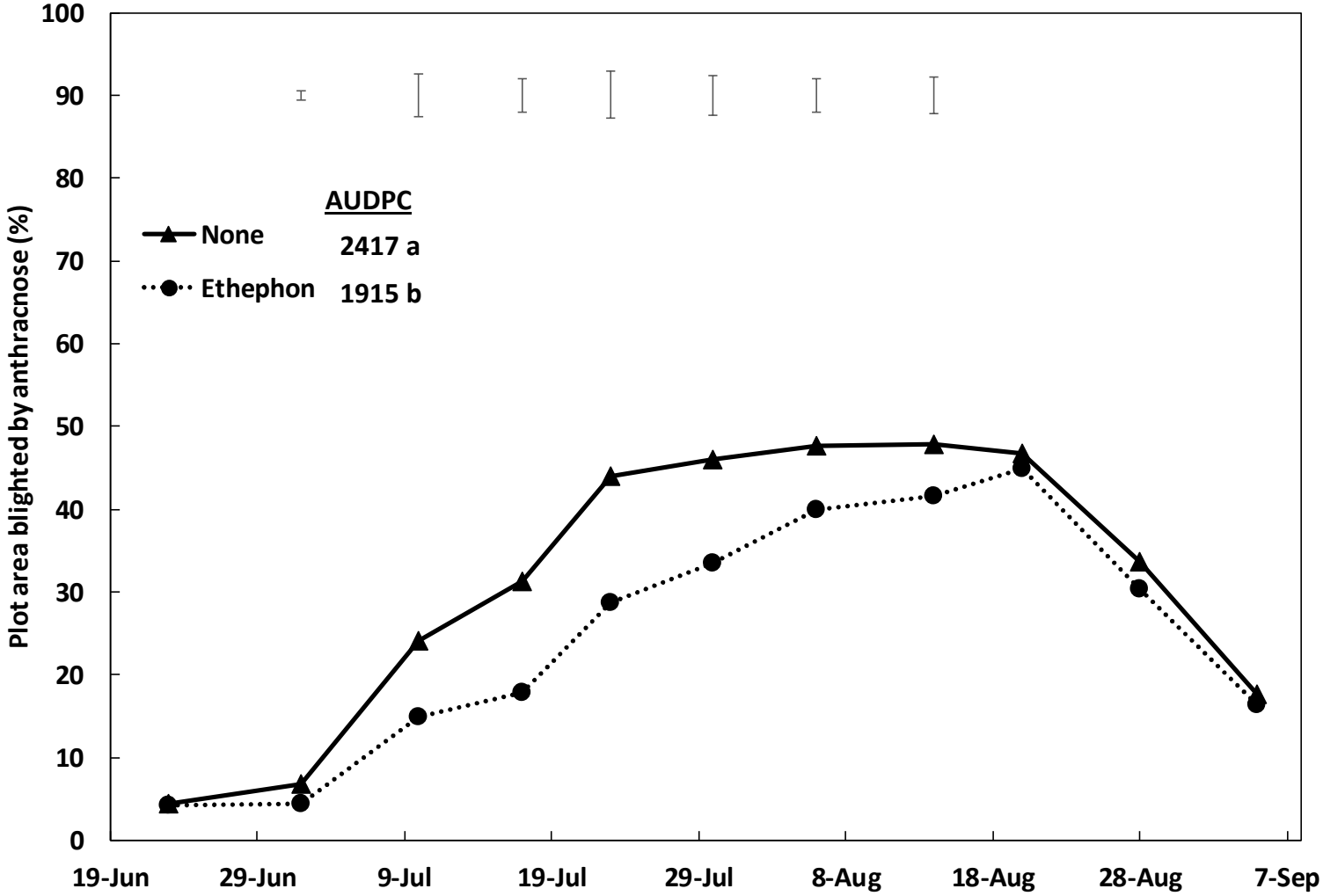

Figure 3. Anthracnose severity influenced by trinexapac-ethyl treatment interval on annual bluegrass putting green turf in Storrs, CT during 2014. Trinexapac-ethyl was applied every 14 d or every 200 GDD from 10 April to 27 August 2014 at 0.05 kg a.i. ha^{-1}.

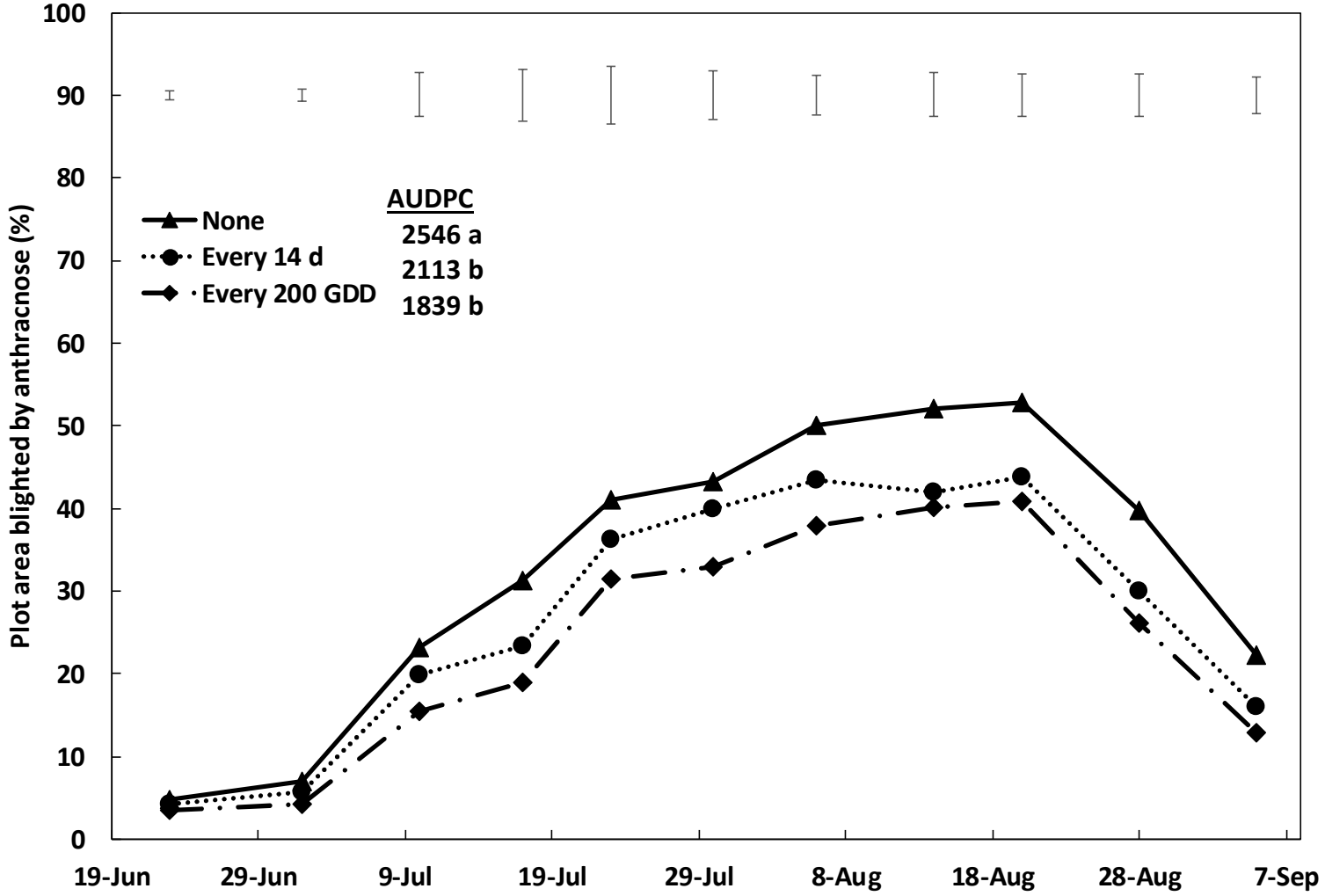

Table 2. Analysis of variance for anthracnose severity affected by seasonal nitrogen fertilization, ethephon and trinexapac-ethyl for annual bluegrass putting green turf in Storrs, CT during 2015.

	Anthracnose Severity												
Source of variation	26 June	3 July	9 July	17 July	24 July	31 July	7 Aug	14 Aug	21 Aug	28 Aug	4 Sept	12 Sept	AUDPC[†]
	---------- Significant level ----------												
Seasonal Nitrogen (N)[‡]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Ethephon (ET) [§]	NS	NS	*	NS	NS	NS	*	NS	*	**	*	NS	NS
Trinexapac-ethyl (TE)[¶]	NS	***	NS	**	NS	**	*	**	***	***	***	***	***
N x ET	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
N x TE	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
ET x TE	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
N x ET x TE	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV, %	54.3	37.7	38.4	32.8	24.6	21.4	23.3	22.1	18.9	19.8	25.1	35.2	19.2

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively; NS Not significant at p>0.05.

[†] Area under the disease progress curve.

[‡] Nitrogen treatments were allocated seasonally emphasizing spring + fall, and spring application timings. Spring + Fall N program was applied as a split application of 18.3 kg N a.i. ha^{-1} on 16 Sept. and 2 Oct. 2014 and a split application of 6.1 kg N a.i. ha^{-1} on 17 Apr. and 1 May 2015. Spring N was applied as a split application of 24.4 kg N a.i. ha^{-1} on 17 Apr. and 1 May 2015.

[§] Ethephon was applied as a split application of 3.8 kg a.i. ha^{-1} on 16 Apr. and 8 May 2015.

[¶] Trinexapac-ethyl was applied every 14 d from 16 Apr. to 10 Sept. 2015 at 0.05 kg a.i. ha^{-1}.

Figure 4. Anthracnose severity influenced by seasonal nitrogen fertilization on annual bluegrass putting green turf in Storrs, CT during 2015. Nitrogen treatments were allocated seasonally emphasizing spring + fall, and spring application timings. Spring + Fall N program was applied as a split application of 18.3 kg N a.i. ha^{-1} on 16 Sept. and 2 Oct. 2014 and a split application of 6.1 kg N a.i. ha^{-1} on 17 Apr. and 1 May 2015. Spring N was applied as a split application of 24.4 kg N a.i. ha^{-1} on 17 Apr. and 1 May 2015.

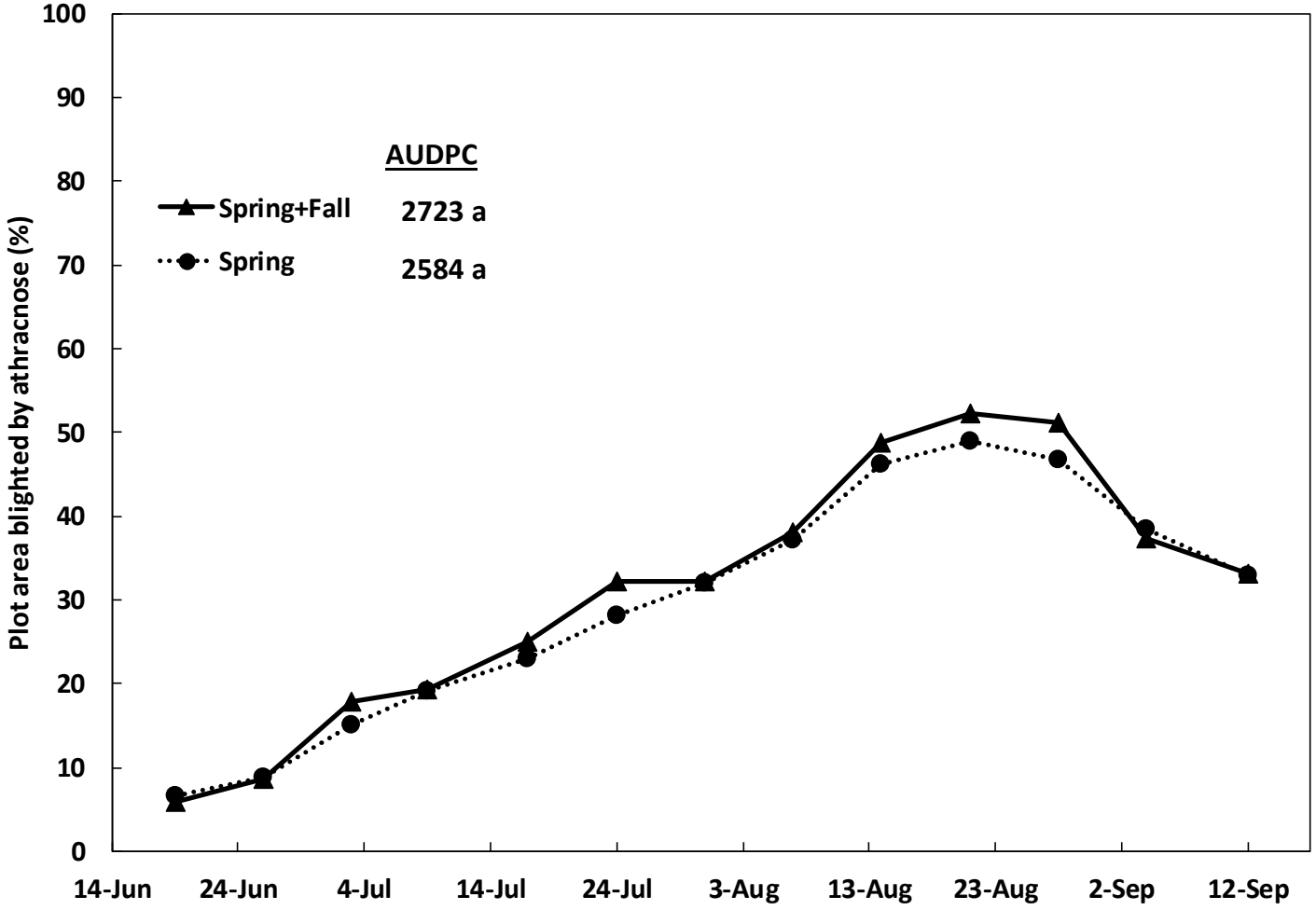

Figure 5. Anthracnose severity influenced by ethephon on annual bluegrass putting green turf in Storrs, CT during 2015. Ethephon was applied as a split application of 3.8 kg a.i. ha^{-1} on 16 Apr. and 8 May 2015.

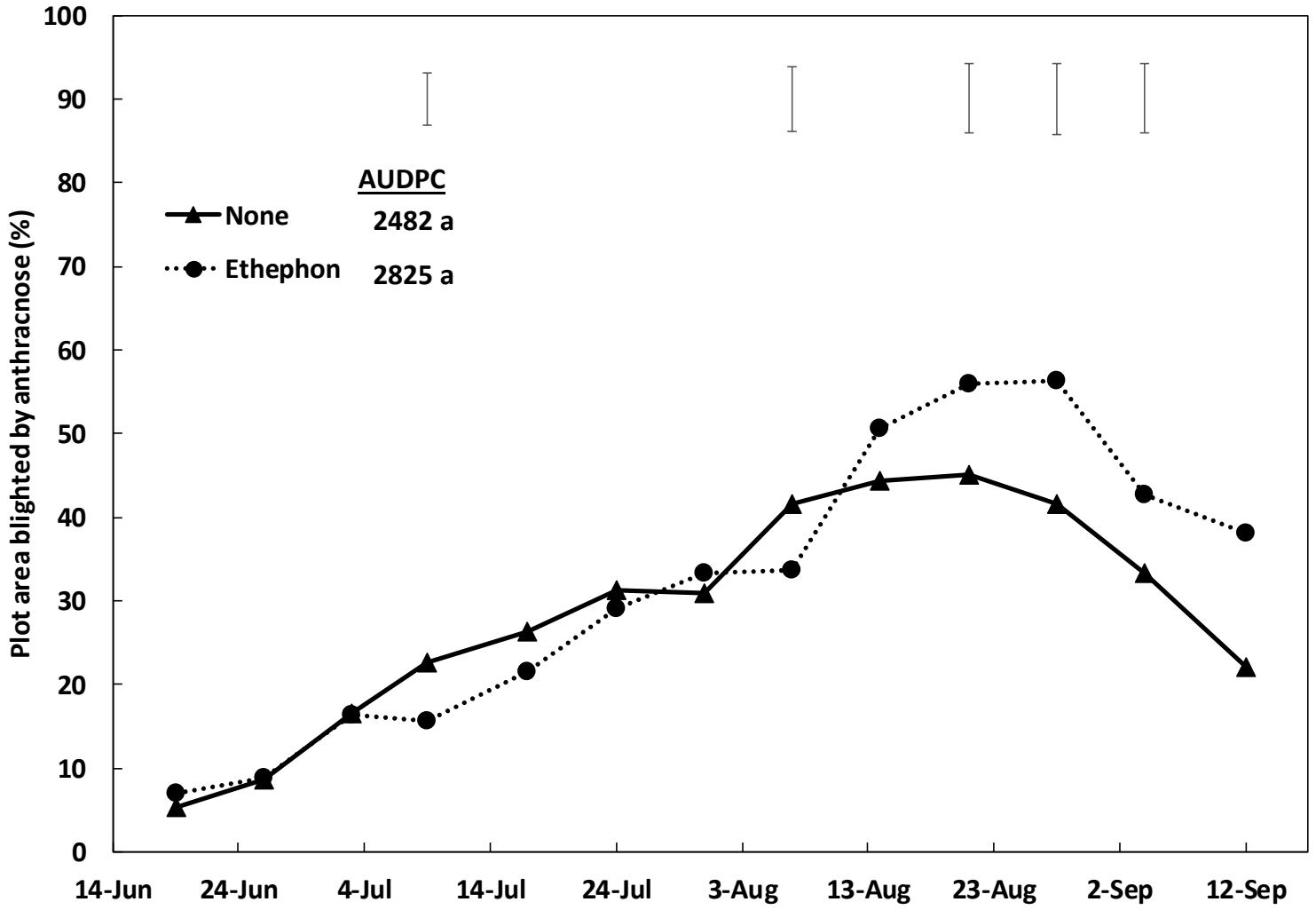

Figure 6. Anthracnose severity influenced by trinexapac-ethyl on annual bluegrass putting green turf in Storrs, CT during 2015. Trinexapac-ethyl was applied every 14 d from 16 Apr. to 10 Sept. 2015 at 0.05 kg a.i. ha^{-1}.

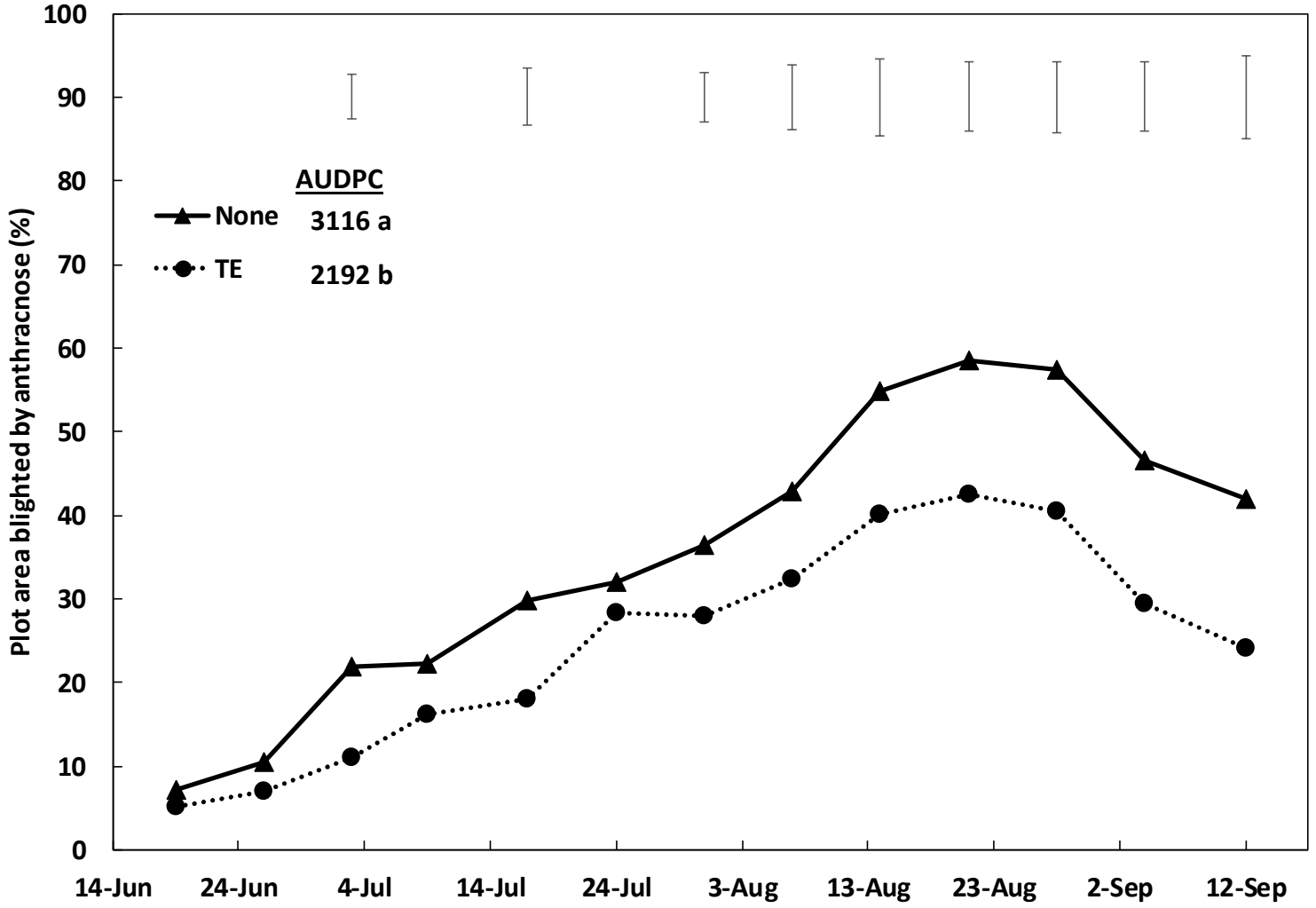

Table 3. Analysis of variance for turf quality ratings as affected by seasonal nitrogen fertilization, ethephon and trinexapac-ethyl interval for annual bluegrass putting green turf in Storrs, CT during 2014 and 2015, plus source effects for the ANOVA.

	Turf quality									
	2014					2015				
Main effects	2 May	28 May	24 June	24 July	28 Aug	7 May	4 June	3 July	6 Aug	4 Sept
Seasonal Nitrogen[†]	---------- 1 to 9 (9=best) ----------									
Fall	5.3 b	4.9 c	5.4 b	4.2	4.3	ND[¶]	ND	ND	ND	ND
Spring + Fall	5.8 a	5.4 b	5.8 a	4.5	4.6	6.5 b	7.1	4.4	4.0	4.6
Spring	6.2 a	6.4 a	6.1 a	4.7	4.6	7.5 a	6.9	4.8	4.1	4.5
Ethephon[‡]										
None	5.9	5.4 b	5.3 b	3.9 b	4.4	6.8	7.3	4.3	4.2	5.0 a
7.6 kg a.i. ha^{-1}	5.6	5.7 a	6.2 a	5.1 a	4.6	7.1	6.7	4.8	3.9	4.1 b
Trinexapac-ethyl Interval[§]										
None	5.4 b	5.2 b	5.6	4.1 b	4.0 b	6.9	7.1	4.1 b	3.6 b	4.0 b
Every 14 d	5.7 b	5.6 a	5.6	4.5 ab	4.6 a	7.0	6.9	5.1 a	4.5 a	5.2 a
Every 200 GDD	6.2 a	5.8 a	6.0	4.8 a	4.9 a	ND	ND	ND	ND	ND
Source of variation	---------- ANOVA ----------									
Seasonal Nitrogen (N)	***	***	**	NS	NS	**	NS	NS	NS	NS
Ethephon (ET)	NS	***	***	***	NS	NS	NS	NS	NS	*
Trinexapac-ethyl Interval (TE)	**	***	NS	*	***	NS	NS	***	**	***
N x ET	NS	***	*	NS	NS	NS	NS	NS	NS	NS
N x TE	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
ET x TE	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
N x ET x TE	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV, %	13.2	6.4	12.2	19.6	16.0	9.4	21.1	18.4	14.3	21.7

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively; NS Not significant at p>0.05.

[†] Nitrogen treatments were allocated seasonally emphasizing fall, spring + fall, and spring application timings. Fall N was applied as a split application of 24.4 kg on 15 Oct. and 7 Nov. 2013. Spring + Fall N timings were applied as a split application of 18.3 kg ha^{-1} on 15 Oct. and 7 Nov. 2013 and a split application of 6.1 kg ha^{-1} on 11 and 25 Apr. 2014; a split application of 18.3 kg ha^{-1} on 16 Sept. and 2 Oct. 2014 and a split application of 6.1 kg ha^{-1} on 17 Apr. and 1 May 2015. Spring N was applied as a split application of 24.4 kg ha^{-1} on 11 and 25 Apr. 2014, and 17 Apr. and 1 May 2015.

[‡] Ethephon was applied as a split application of 3.8 kg a.i. ha^{-1} on 10 and 25 Apr. 2014; and 16 Apr. and 8 May 2015.

§ Trinexapac-ethyl was applied every 14 d or every 200 GDD from 10 Apr. to 27 Aug. and every 14 d from 16 Apr. to 10 Sept. 2015 at 0.05 kg a.i. ha^{-1}.
¶ Not determined; this treatment was omitted from the study in 2015 due to reduced available treatment area following winter injury.
Means in a column for a specific mean effects followed by the same letter are not significant different according to Fisher LSD (α=0.05).

Table 4. Turf quality ratings as affected by seasonal nitrogen fertilization and ethephon for annual bluegrass putting green turf in Storrs, CT during 2014.

	Turf quality			
	28 May		24 June	
	Ethephon[‡]			
Seasonal Nitrogen[†]	None	7.6 kg a.i. ha^{-1}	None	7.6 kg a.i. ha^{-1}
	1 to 9 (9=best)			
Fall	4.8 cA[§]	5.0 cA	5.1 aB	5.7 bA
Spring + Fall	5.4 bA	5.4 bA	5.4 aB	6.2 bA
Spring	6.0 aB	6.8 aA	5.3 aB	6.8 aA
LSD	0.3		0.5	

[†] Nitrogen treatments were allocated seasonally emphasizing fall, spring + fall, and spring application timings. Fall N was applied as a split application of 24.4 kg ha^{-1} on 15 Oct. and 7 Nov. 2013, Spring + Fall N timings were applied as a split application of 18.3 kg ha^{-1} on 15 Oct. and 7 Nov. 2013 and a split application of 6.1 kg ha^{-1} on 11 and 25 Apr. 2014, and spring N was applied as a split application of 24.4 kg ha^{-1} on 11 and 25 Apr. 2014.

[‡] Ethephon was applied as a split application of 3.8 kg a.i. ha^{-1} on 10 and 25 Apr. 2014.

[§] Lower case letters separated means in columns; and upper case letters separated means in rows followed by the same letters are not significant different according to Fisher's LSD (α=0.05).

Table 5. Analysis of variance for seedhead production affected by seasonal nitrogen fertilization, ethephon and trinexapac-ethyl interval for annual bluegrass putting green turf in Storrs, CT during 2014 and 2015, plus source effects for the ANOVA.

	Seedhead expression							
	2014				2015			
Main effects	6 May	19 May	2 June	16 June	18 May	3 June	17 June	30 June
Seasonal Nitrogen[†]	%							
Fall	1.6	43.5 a	32.3 b	5.9 b	ND[¶]	ND	ND	ND
Spring + Fall	1.9	42.1 a	32.9 b	4.8 b	10.4	29.0 b	29.3	10.8
Spring	1.5	35.1 b	39.8 a	11.5 a	12.1	37.9 a	29.1	11.8
Ethephon[‡]								
None	2.7 a	54.4 a	49.9 a	12.4 a	15.4 a	55.0 a	53.8 a	18.8 a
7.6 kg a.i. ha^{-1}	0.6 b	26.0 b	20.1 b	2.3 b	7.1 b	11.9 b	4.7 b	3.8 b
Trinexapac-ethyl Interval[§]								
None	2.6 a	46.7 a	35.0	5.9	12.1	34.6	31.0	9.9
Every 14 d	1.6 b	37.8 b	35.4	8.1	10.4	32.3	27.4	12.6
Every 200 GDD	1.0 b	36.3 b	34.6	8.1	ND	ND	ND	ND
Source of variation	ANOVA							
Seasonal Nitrogen (N)	NS	***	***	***	NS	**	NS	NS
Ethephon (ET)	***	***	***	***	***	***	***	***
Trinexapac-ethyl Interval (TE)	***	***	NS	NS	NS	NS	NS	NS
N x ET	NS	*	***	***	NS	**	NS	NS
N x TE	NS	NS	NS	NS	NS	NS	NS	NS
ET x TE	NS	NS	NS	NS	NS	NS	NS	NS
N x ET x TE	NS	NS	NS	NS	NS	NS	NS	NS
CV, %	76.5	17.2	16.1	19.6	31.4	17.6	33.0	50.0

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively; NS Not significant at p>0.05.

[†] Nitrogen treatments were allocated seasonally emphasizing fall, spring + fall, and spring application timings. Fall nitrogen was applied as a split application of 24.4 kg on 15 Oct. and 7 Nov. 2013. Spring + Fall nitrogen timings were applied as a split application of 18.3 kg ha^{-1} on 15 Oct. and 7 Nov. 2013 and a split application of 6.1 kg ha^{-1} on 11 and 25 Apr. 2014; a split application of 18.3 kg ha^{-1} on 16 Sept. and 2 Oct. 2014 and a split application of 6.1 kg ha^{-1} on 17 Apr. and 1 May 2015. Spring nitrogen was applied as a split application of 24.4 kg ha^{-1} on 11 and 25 Apr. 2014, and 17 Apr. and 1 May 2015.

[‡] Ethephon was applied as a split application of 3.8 kg a.i. ha^{-1} on 10 and 25 Apr. 2014, 16 Apr. and 8 May 2015.

§ Trinexapac-ethyl was applied every 14 d or every 200 GDD from 10 Apr. to 27 Aug. and every 14 d from 16 Apr. to 10 Sept. 2015 at 0.05 kg a.i. ha^{-1}.
¶ Not determined; this treatment was omitted from the study in 2015 due to reduced available treatment area following winter injury.
Means in a column for a specific mean effects followed by the same letter are not significant different according to Fisher LSD (α=0.05).

Table 6. Seedhead production affected by seasonal nitrogen fertilization and ethephon for annual bluegrass putting green turf in Storrs, CT during 2014 and 2015.

	Seedhead expression							
	19 May 2014		2 June 2014		16 June 2014		3 June 2015	
	Ethephon‡							
Seasonal Nitrogen†	None	7.6 kg a.i. ha^{-1}	None	7.6 kg a.i. ha^{-1}	None	7.6 kg a.i. ha^{-1}	None	7.6 kg a.i. ha^{-1}
	%							
Fall	55.2 aA§	31.7 aB	42.1 bA	22.5 aB	10.3 bA	1.4 aB	ND¶	ND
Spring + Fall	55.4 aA	22.8 aB	45.0 bA	20.8 abB	8.2 bA	1.3 aB	46.7 bA	11.3 aB
Spring	52.5 aA	17.7 bB	60.5 aA	17.1 bB	18.8 aA	4.2 aB	63.3 aA	12.5 aB
LSD	5.7		4.4		3.3		7.6	

† Nitrogen treatments were allocated seasonally emphasizing fall, spring + fall, and spring application timings. Fall N was applied as a split application of 24.4 kg on 15 Oct. and 7 Nov. 2013. Spring + Fall N timings were applied as a split application of 18.3 kg ha^{-1} on 15 Oct. and 7 Nov. 2013 and a split application of 6.1 kg ha^{-1} on 11 and 25 Apr. 2014; a split application of 18.3 kg ha^{-1} on 16 Sept. and 2 Oct. 2014 and a split application of 6.1 kg ha^{-1} on 17 Apr. and 1 May 2015. Spring N was applied as a split application of 24.4 kg ha^{-1} on 11 and 25 Apr. 2014, and 17 Apr. and 1 May 2015.

‡ Ethephon was applied as a split application of 3.8 kg a.i. ha^{-1} on 10 and 25 Apr. 2014, 16 Apr. and 8 May 2015.

§ Lower case letters separated means in columns; and upper case letters separated means in rows followed by the same letters are not significant different according to Fisher's LSD (α=0.05).

¶ Not determined; this treatment was omitted from the study in 2015 due to reduced available treatment area following winter injury.

Table 7. Analysis of variance for clipping yield affected by seasonal nitrogen fertilization, ethephon and trinexapac-ethyl interval for annual bluegrass putting green turf in Storrs, CT during 2014, plus source effects for the ANOVA.

	Clipping yield				
Main effects	7 May	19 May	2 June	16 June	30 June
Seasonal Nitrogen[†]	---------- g m^{-2} ----------				
Fall	3.01 b	5.32	3.01	2.37 b	1.99 b
Spring + Fall	3.14 ab	5.11	2.89	2.35 b	2.11 b
Spring	3.42 a	5.66	3.13	2.96 a	2.48 a
Ethephon[‡]					
None	2.39 b	5.05 b	3.43 a	2.74 a	2.14
7.6 kg a.i. ha^{-1}	3.99 a	5.68 a	2.58 b	2.39 b	2.25
Trinexapac-ethyl Interval[§]					
None	3.61 a	6.61 a	3.54 a	2.77 a	2.63 a
Every 14 d	3.06 b	5.23 b	3.10 a	2.48 b	2.09 b
Every 200 GDD	2.90 c	4.25 c	2.39 b	2.44 b	1.87 b
Source of variation	---------- ANOVA ----------				
Seasonal Nitrogen (N)	*	NS	NS	***	***
Ethephon (ET)	***	*	***	**	NS
Trinexapac-ethyl Interval (TE)	***	***	***	*	***
N x ET	NS	*	NS	NS	NS
N x TE	NS	NS	NS	NS	NS
ET x TE	NS	NS	NS	NS	NS
N x ET x TE	NS	NS	NS	NS	NS
CV, %	19.0	27.4	40.1	19.5	24.7

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively; NS Not significant at p>0.05.

[†] Nitrogen treatments were allocated seasonally emphasizing fall, spring + fall, and spring application timings. Fall nitrogen was applied as a split application of 24.4 kg ha^{-1} on 15 Oct. and 7 Nov. 2013, Spring + Fall nitrogen timings were applied as a split application of 18.3 kg ha^{-1} on 15 Oct. and 7 Nov. 2013 and a split application of 6.1 kg ha^{-1} on 11 and 25 Apr. 2014, and spring nitrogen was applied as a split application of 24.4 kg ha^{-1} on 11 and 25 Apr. 2014.

[‡] Ethephon was applied as a split application of 3.8 kg a.i. ha^{-1} on 10 and 25 Apr. 2014.

§ Trinexapac-ethyl was applied every 14 d or every 200 GDD at 0.05 kg a.i. ha^{-1}.
Means in a column for a specific mean effects followed by the same letter are not significant different according to Fisher LSD (α=0.05).

Table 8. Analysis of variance for clipping yield affected by seasonal nitrogen fertilization, ethephon and trinexapac-ethyl for annual bluegrass putting green turf in Storrs, CT during 2015, plus source effects for the ANOVA.

	Clipping yield			
Main effects	18 May	1 June	18 June	29 June
Seasonal Nitrogen[†]	---------- g m^{-2} ----------			
Spring + Fall	6.77 b	5.59	6.23	5.97
Spring	9.66 a	6.06	6.08	6.28
Ethephon (ET)[‡]				
None	6.38 b	6.27 a	7.00	6.06
7.6 kg a.i. ha^{-1}	10.06 a	5.38 b	5.30	6.21
Trinexapac-ethyl[§]				
None	8.79	5.82	6.35	6.01
Every 14 d	7.65	5.83	5.94	6.24
Source of variation	---------- ANOVA ----------			
Seasonal Nitrogen (N)	**	NS	NS	NS
Ethephon (ET)	***	**	NS	NS
Trinexapac-ethyl (TE)	NS	NS	NS	NS
N x ET	NS	NS	NS	NS
N x TE	NS	*	NS	NS
ET x TE	NS	NS	NS	NS
N x ET x TE	NS	NS	NS	NS
CV, %	24.2	12.7	33.5	19.3

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively; NS Not significant at p>0.05.

[†] Nitrogen treatments were allocated seasonally emphasizing spring + fall, and spring application timings. Spring + Fall N program was applied as a split application of 18.3 kg N a.i. ha^{-1} on 16 Sept. and 2 Oct. 2014 and a split application of 6.1 kg N a.i. ha^{-1} on 17 Apr. and 1 May 2015. Spring N was applied as a split application of 24.4 kg N a.i. ha^{-1} on 17 Apr. and 1 May 2015.

[‡] Ethephon was applied as a split application of 3.8 kg a.i. ha^{-1} on 16 Apr. and 8 May 2015.

[§] Trinexapac-ethyl was applied at 0.05 kg a.i. ha^{-1} every 14 d from 16 Apr. to 10 Sept. 2015.

Table 9. Clipping yield affected by seasonal nitrogen fertilization and ethephon for annual bluegrass putting green turf in Storrs, CT during 2014 and 2015.

	Clipping yield			
	19 May 2014		1 June 2015	
	Ethephon‡			
Seasonal Nitrogen†	None	7.6 kg a.i. ha^{-1}	None	7.6 kg a.i. ha^{-1}
	---------- g m^{-2} ----------			
Fall	5.34 aA§	5.23 bA	ND¶	ND
Spring + Fall	4.83 aA	5.34 bA	5.98 aA	5.15 bA
Spring	4.89 aB	6.37 aA	5.57 aA	6.44 aA
LSD	0.89		0.91	

† Nitrogen treatments were allocated seasonally emphasizing fall, spring + fall, and spring application timings. Fall N was applied as a split application of 24.4 kg on 15 Oct. and 7 Nov. 2013. Spring + Fall N timings were applied as a split application of 18.3 kg ha^{-1} on 15 Oct. and 7 Nov. 2013 and a split application of 6.1 kg ha^{-1} on 11 and 25 Apr. 2014; a split application of 18.3 kg ha^{-1} on 16 Sept. and 2 Oct. 2014 and a split application of 6.1 kg ha^{-1} on 17 Apr. and 1 May 2015. Spring N was applied as a split application of 24.4 kg ha^{-1} on 11 and 25 Apr. 2014, and 17 Apr. and 1 May 2015.

‡ Ethephon was applied as a split application of 3.8 kg a.i. ha^{-1} on 10 and 25 Apr. 2014, 16 Apr. and 8 May 2015.

§ Lower case letters separated means in columns; and upper case letters separated means in rows followed by the same letters are not significant different according to Fisher's LSD (α=0.05).

¶ Not determined; this treatment was omitted from the study in 2015 due to reduced available treatment area following winter injury.

Table 10. Analysis of variance for clipping nitrogen concentration affected by seasonal nitrogen fertilization, ethephon and trinexapac-ethyl interval for annual bluegrass putting green turf in Storrs, CT during 2014, plus source effects for the ANOVA.

	Clipping nitrogen concentration									
Main effects	7 May	19 May	2 June	16 June	30 June	21 July	29 July	11 Aug	25 Aug	20 Oct
Seasonal Nitrogen[†]	---------- g kg^{-1} ----------									
Fall	22.6 c	23.5 c	26.6 b	32.7 b	32.8 b	39.8	36.8	39.5	40.4	34.0 a
Spring + Fall	24.1 b	25.3 b	27.5 a	34.1 a	34.4 a	40.4	37.8	40.1	40.9	31.9 a
Spring	28.3 a	29.4a	27.5 a	34.3 a	34.1ab	40.2	37.6	39.5	40.3	23.3 b
Ethephon[‡]										
None	25.7 a	25.5 b	26.8 b	33.5	33.0 b	40.1	36.3 b	39.1 b	40.4	29.5 a
7.6 kg a.i. ha^{-1}	24.6 b	26.7 a	27.6 a	33.9	34.5 a	40.2	38.5 a	40.3 a	40.7	25.7 b
Trinexapac-ethyl Interval[§]										
None	24.2 b	25.1 b	26.9	32.9 b	33.3	39.6	36.3 b	39.1	39.8	27.6
Every 14 d	25.7 a	26.3 a	27.2	33.9 ab	33.5	40.2	37.5 ab	39.7	40.5	27.6
Every 200 GDD	25.5 a	26.8 a	27.6	34.4 a	34.5	40.6	38.4 a	40.3	41.3	27.5
Source of variation	ANOVA									
Seasonal Nitrogen (N)	***	***	**	**	*	NS	NS	NS	NS	***
Ethephon (ET)	***	**	***	NS	**	NS	***	*	NS	*
Trinexapac-ethyl Interval (TE)	***	***	NS	*	NS	NS	*	NS	NS	NS
N x ET	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
N x TE	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
ET x TE	NS	NS	**	NS	NS	NS	NS	NS	NS	NS
N x ET x TE	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV, %	4.7	6.7	4.2	5.9	6.8	4.4	7.4	5.8	6.3	12.1

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively; NS Not significant at p>0.05.

[†] Nitrogen treatments were allocated seasonally emphasizing fall, spring + fall, and spring application timings. Fall nitrogen was applied as a split application of 24.4 kg ha^{-1} on 15 Oct. and 7 Nov. 2013, Spring + Fall N timings were applied as a split application of 18.3 kg ha^{-1} on 15 Oct. and 7 Nov. 2013 and a split application of 6.1 kg ha^{-1} on 11 and 25 Apr. 2014, and spring N was applied as a split application of 24.4 kg ha^{-1} on 11 and 25 Apr. 2014.

[‡] Ethephon was applied as a split application of 3.8 kg a.i. ha^{-1} on 10 and 25 Apr. 2014.

[§] Trinexapac-ethyl was applied every 14 d or every 200 GDD at 0.05 kg a.i. ha^{-1}.

¶ Not determined.
Means in a column for a specific mean effects followed by the same letter are not significant different according to Fisher LSD (α=0.05).

Table 11. Analysis of variance for clipping nitrogen concentration affected by seasonal nitrogen fertilization, ethephon and trinexapac-ethyl for annual bluegrass putting green turf in Storrs, CT during 2015, plus source effects for the ANOVA.

	Clipping nitrogen concentration						
Main effects	18 May	1 June	18 June	29 June	13 July	10 Aug	27 Aug
Seasonal Nitrogen[†]	---------- g kg^{-1} ----------						
Spring + Fall	14.3 b	21.1	23.1	21.7	27.7	33.1	30.9
Spring	21.0 a	21.6	23.0	22.1	27.4	33.2	31.0
Ethephon (ET)[‡]							
None	18.7	21.9	23.3	23.4 a	26.9	32.4	31.6
7.6 kg a.i. ha^{-1}	16.7	20.6	22.8	20.3 b	28.2	32.9	30.3
Trinexapac-ethyl[§]							
None	17.6	20.9	22.8	20.9	27.0	32.6	29.7 b
Every 14 d	17.8	21.6	23.3	22.9	28.1	33.7	32.2 a
Source of variation	---------- ANOVA ----------						
Seasonal Nitrogen (N)	***	NS	NS	NS	NS	NS	NS
Ethephon (ET)	NS	NS	NS	**	NS	NS	NS
Trinexapac-ethyl (TE)	NS	NS	NS	NS	NS	NS	**
N x ET	NS	NS	NS	NS	NS	NS	NS
N x TE	NS	NS	NS	NS	NS	*	NS
ET x TE	NS	NS	NS	NS	NS	NS	NS
N x ET x TE	NS	NS	NS	NS	NS	NS	NS
CV, %	24.2	12.7	33.5	19.3	24.2	20.8	35.6

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively; NS Not significant at p>0.05.

[†] Nitrogen treatments were allocated seasonally emphasizing spring + fall, and spring application timings. Spring + Fall nitrogen program was applied as a split application of 18.3 kg N a.i. ha^{-1} on 16 Sept. and 2 Oct. 2014 and a split application of 6.1 kg N a.i. ha^{-1} on 17 Apr. and 1 May 2015. Spring nitrogen was applied as a split application of 24.4 kg N a.i. ha^{-1} on 17 Apr. and 1 May 2015.

[‡] Ethephon was applied as a split application of 3.8 kg a.i. ha^{-1} on 16 Apr. and 8 May 2015.

[§] Trinexapac-ethyl was applied at 0.05 kg a.i. ha^{-1} every 14 d from 16 Apr. to 10 Sept. 2015.

Table 12. Analysis of variance for anthracnose severity affected by trinexapac-ethyl and prohexdione-Ca for annual bluegrass putting green turf in Storrs, CT during 2015, plus source effects for the ANOVA. Trinexapac-ethyl (TE) was applied every 14 d or every 200 GDD from 4 June through 12 Sept. 2015 at 0.05 kg a.i. ha^{-1}. Prohexdione-Ca (PC) was applied every 14 d or every 300 GDD from 4 June through 10 Sept. 2015 at 0.08 kg a.i. ha^{-1}.

	Anthracnose Severity											
Treatments	6 July	15 July	22 July	28 July	5 Aug	12 Aug	19 Aug	25 Aug	1 Sept	9 Sept	16 Sept	AUDPC†
Main treatments						%						
Untreated	13.4	19.2 a	24.3 a	47.8 a	60.9 a	67.3 a	73.0 a	70.7 a	69.6 a	64.7 a	62.4 a	599.90 a
TE, every 14 d	14.8	15.8 ab	20.1 ab	35.6 b	43.9 b	50.7 b	60.7 b	59.8 b	59.0 b	52.6 b	56.1 ab	454.35 b
TE, every 200 GDD	10.6	13.8 b	14.6 c	33.2 b	36.9 c	49.6 bc	52.0 c	55.0 b	54.6 b	47.3 bc	48.6 cd	405.45 c
PC, every 14 d	13.6	14.0 b	19.1 b	29.9 b	42.7 b	43.6 c	57.1 bc	54.6 b	57.9 b	50.7 b	53.6 bc	423.20 bc
PC, every 300 GDD	12.0	19.8 a	17.0 bc	34.2 b	36.0 c	43.5 c	50.2 c	53.7 b	55.4 b	44.6 c	44.1 d	398.32 c
Untreated	13.4	19.2	24.3	47.8	60.9	67.3	73.0	70.7	69.6	64.7	62.4	600
Plant growth regulators	12.7	15.9	17.7	33.2	39.9	46.8	55.0	55.8	56.7	48.8	50.6	420
TE	12.7	14.8	17.4	34.3	43.4	50.1	56.4	56.9	56.8	50.0	52.4	430
PC	12.8	16.9	24.3	32.0	39.4	43.5	53.6	54.1	56.7	48.6	48.9	411
14 d interval	14.2	14.9	19.6	32.7	43.3	47.1	58.9	57.1	58.5	51.7	53.4	439
GDD interval	11.3	16.8	15.9	33.6	36.5	46.5	51.1	54.4	55.0	45.9	46.4	402
Source of variation						ANOVA						
Main treatments	NS	*	**	***	***	***	***	***	**	***	***	***
Untreated *vs* PGRs‡	NS	NS	***	***	***	***	***	***	***	***	***	***
TE *vs* PC§	NS	NS	NS	NS	NS	**	NS	NS	NS	NS	NS	NS
Calendar *vs* GDD ¶	NS	NS	*	NS	**	NS	**	NS	NS	**	**	**
PGR x interval interaction	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively; NS Not significant at p>0.05.

† Area under the disease progress curve.

‡ Comparison of: untreated vs. TE, 14 d; TE, 200 GDD; PC, 14 d; and PC, 300 GDD.

§ Comparison of: TE, 14 d and TE, 200 GDD vs. PC, 14 d and PC, 300 GDD

¶ Comparison of: TE, 14 d and PC, 14 d vs. TE, 200 GDD and PC 300 GDD

Means in a column for a specific mean effects followed by the same letter are not significant different according to Fisher LSD (α=0.05).

Figure 7. Anthracnose severity influenced by trinexapac-ethyl, prohexadione-Ca and treatment interval on annual bluegrass putting green turf in Storrs, CT during 2015. Trinexapac-ethyl (TE) was applied every 14 d or every 200 GDD from 4 June through 12 Sept. 2015 at 0.05 kg a.i. ha^{-1}. Prohexdione-Ca (PC) was applied every 14 d or every 300 GDD from 4 June through 10 Sept. 2015 at 0.08 kg a.i. ha^{-1}.

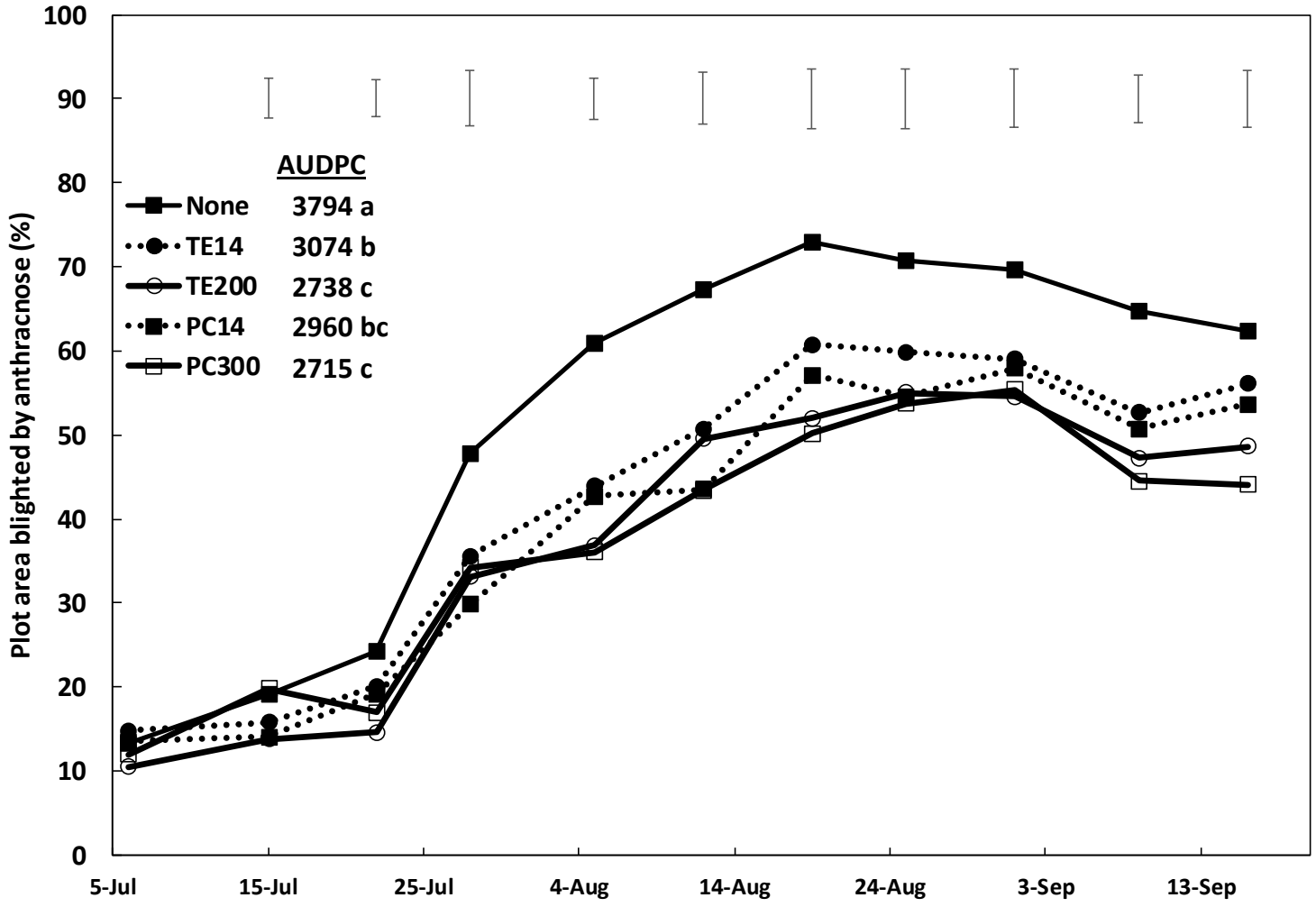

Table 13. Analysis of variance for turf quality affected by trinexapac-ethyl and prohexdione-Ca for annual bluegrass putting green turf in Storrs, CT during 2015, plus source effects for the ANOVA. Trinexapac-ethyl (TE) was applied every 14 d or every 200 GDD from 4 June through 12 Sept. 2015 at 0.05 kg a.i. ha^{-1}. Prohexdione-Ca (PC) was applied every 14 d or every 300 GDD from 4 June through 10 Sept. 2015 at 0.08 kg a.i. ha^{-1}.

	Turf quality					
Treatments	6 July	22 July	5 Aug	19 Aug	1 Sept	16 Sept
	---------- 1 to 9 (9=best) ----------					
Untreated	4.3	4.0	2.3 c	1.3 b	2.3	2.8 b
TE, every 14 d	4.3	4.3	3.0 b	2.8 a	2.5	3.1 b
TE, every 200 GDD	4.8	4.8	3.8 a	3.3 a	3.3	3.1 b
PC, every 14 d	4.3	4.5	3.5 ab	3.0 a	2.8	3.1 b
PC, every 300 GDD	4.5	4.8	4.0 a	3.5 a	3.8	4.1 a
Untreated	4.3	4.0	2.3	1.3	2.3	2.8
Plant growth regulators	4.5	4.6	3.7	3.2	3.1	3.4
TE	4.6	4.6	3.4	3.1	2.9	3.1
PC	4.4	4.7	3.9	3.3	3.3	3.6
14 d interval	4.3	4.4	3.4	2.9	2.7	3.1
GDD interval	4.7	4.8	3.9	3.4	3.6	3.6
Source of variation	---------- ANOVA ----------					
Main treatments	NS	NS	**	***	NS	*
Untreated *vs* PGRs[†]	NS	*	***	***	NS	NS
TE *vs* PC[‡]	NS	NS	NS	NS	NS	NS
Calendar *vs* GDD [§]	NS	NS	*	NS	*	NS
PGR x interval interaction	NS	NS	NS	NS	NS	NS

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively; NS Not significant at p>0.05.

[†] Comparison of: untreated vs. TE, 14 d; TE, 200 GDD; PC, 14 d; and PC, 300 GDD.

[‡] Comparison of: TE, 14 d and TE, 200 GDD vs. PC, 14 d and PC, 300 GDD

[§] Comparison of: TE, 14 d and PC, 14 d vs. TE, 200 GDD and PC 300 GDD

Means in a column for a specific mean effects followed by the same letter are not significant different according to Fisher LSD (α=0.05).

Table 14. Analysis of variance for clipping yield affected by trinexapac-ethyl and prohexdione-Ca for annual bluegrass putting green turf in Storrs, CT during 2015, plus source effects for the ANOVA. Trinexapac-ethyl (TE) was applied every 14 d or every 200 GDD from 4 June through 12 Sept. 2015 at 0.05 kg a.i. ha^{-1}. Prohexdione-Ca (PC) was applied every 14 d or every 300 GDD from 4 June through 10 Sept. 2015 at 0.08 kg a.i. ha^{-1}.

	Clipping yield		
Plant growth regulators	18 Jun	29 Jun	13 Jul
	---------- g m^{-2} ----------		
Untreated	7.2 a	5.7	9.3 a
TE, every 14 d	6.9 a	5.2	7.5 ab
TE, every 200 GDD	5.7 b	4.6	6.1 b
PC, every 14 d	4.3 c	4.8	6.1 b
PC, every 300 GDD	5.6 b	5.6	5.7 b
Untreated	7.2	5.7	9.3
Plant growth regulators	5.6	5.1	6.4
TE	6.3	4.9	6.8
PC	4.9	5.2	5.9
14 d interval	5.6	5.0	6.8
GDD interval	5.7	5.1	5.9
Source of variation		ANOVA	
Main treatments	***	NS	*
Untreated *vs* PGRs[†]	***	NS	**
TE *vs* PC[‡]	***	NS	NS
Calendar *vs* GDD [§]	NS	NS	NS
PGR x interval interaction	***	NS	NS

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively; NS Not significant at p>0.05.

[†] Comparison of: untreated vs. TE, 14 d; TE, 200 GDD; PC, 14 d; and PC, 300 GDD.

[‡] Comparison of: TE, 14 d and TE, 200 GDD vs. PC, 14 d and PC, 300 GDD

[§] Comparison of: TE, 14 d and PC, 14 d vs. TE, 200 GDD and PC 300 GDD

Means in a column for a specific mean effects followed by the same letter are not significant different according to Fisher LSD (α=0.05).

Table 15. Analysis of variance for clipping nitrogen concentration affected by trinexapac-ethyl and prohexdione-Ca for annual bluegrass putting green turf in Storrs, CT during 2015, plus source effects for the ANOVA. Trinexapac-ethyl (TE) was applied every 14 d or every 200 GDD from 4 June through 12 Sept. 2015 at 0.05 kg a.i. ha^{-1}. Prohexdione-Ca (PC) was applied every 14 d or every 300 GDD from 4 June through 10 Sept. 2015 at 0.08 kg a.i. ha^{-1}.

	Clipping nitrogen concentration				
Plant growth regulators	18 Jun	29 Jun	13 Jul	10 Aug	27 Aug
	---------- g kg^{-1} ----------				
Untreated	16.8	19.4	27.9	22.1	24.2
TE, every 14 d	16.8	19.0	26.3	23.3	27.2
TE, every 200 GDD	16.2	18.9	26.8	22.6	23.6
PC, every 14 d	16.4	20.1	26.1	24.8	23.1
PC, every 300 GDD	16.3	19.0	25.4	25.2	26.6
Untreated	16.8	19.4	27.9	22.1	24.2
Plant growth regulators	16.4	19.3	26.2	24.0	25.1
TE	16.5	19.0	26.6	23.0	25.4
PC	16.4	19.6	25.8	25.0	24.9
14 d interval	16.6	19.6	26.2	24.1	25.2
GDD interval	16.3	19.0	26.1	23.9	25.1
Source of variation	---------- ANOVA ----------				
Main treatments	NS	NS	NS	NS	NS
Untreated *vs* PGRs[†]	NS	NS	NS	NS	NS
TE *vs* PC[‡]	NS	NS	NS	NS	NS
Calendar *vs* GDD [§]	NS	NS	NS	NS	NS
PGR x interval interaction	NS	NS	NS	NS	NS

NS Not significant at p>0.05.

[†] Comparison of: untreated vs. TE, 14 d; TE, 200 GDD; PC, 14 d; and PC, 300 GDD.

[‡] Comparison of: TE, 14 d and TE, 200 GDD vs. PC, 14 d and PC, 300 GDD

[§] Comparison of: TE, 14 d and PC, 14 d vs. TE, 200 GDD and PC 300 GDD